Norwegian University of Life Sciences
Faculty of Biosciences
Department of Animal and Aquacultural Sciences

Philosophiae Doctor (PhD)
Thesis 2021:15

Improved starch and protein utilization by extruded feed pellets targeted to benefit dynamics of rumen digestion in dairy cows

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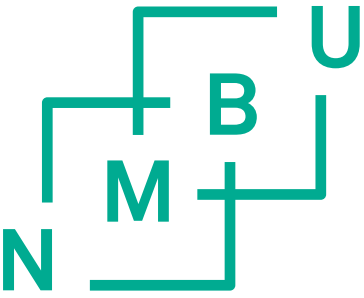

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“In the name of God, the Most Gracious, the Most Merciful”

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List of papers

I. Khan, G. Q., Miladinovic, D. D., Niu, P., Weurding, E., Hees, J. V., Grøseth, M., Prestløkken, E. 2021. Targeting nutrient utilization in ruminant diets through extruder processing: Production and measurement of physical properties of feed pellets. Anim. Feed Sci. Technol. (Submitted)

II. Khan, G. Q., Prestløkken, E., Lund, P., Hellwing, A. L. F., Larsen, M. Effects of density of extruded pellets on starch digestion kinetics, rumen fermentation, fiber digestibility, and enteric methane production in dairy cows. (In manuscript)

III. Khan, G. Q., Larsen, M., Lund, P., Niu, P., Galmeus, D. R. T., Prestløkken, E. Effects of density and fluid stability of extruded barley-soybean meal pellets on digestion kinetics and rumen fermentation pattern in dairy cows. (In manuscript)

Summary

To meet their requirements for energy and amino acids, high-producing dairy cows are fed compound feeds containing high amounts of starch and protein. Optimal utilization of these high-quality feeds is critical, where the rumen digestion represents the main challenge. In Norway, locally grown barley, oats, and wheat are commonly used as energy sources for dairy cows. The rapid rate of rumen starch fermentation of these grains is associated with rumen acidosis and metabolizable energy loss. Similarly, high-quality imported proteins can be subjected to increased rumen degradation and loss of valuable protein. Intestinal digestion of starch and protein is associated with better utilization of metabolizable energy and protein. Thus, shifting a part of starch and protein digestion from the rumen to the small intestine will increase the utilization of feeds.

Traditionally, rumen digestion is targeted by manipulating the rate of rumen degradation through the selection of ingredients and feed processing. However, since rumen digestion results from the concurrent rate of rumen degradation and rate of rumen passage, targeting also passage rate is an alternate strategy to alter rumen digestion behavior, but it is scarcely studied. The density is the main factor governing the rumen passage, where high-density particles have higher rumen outflow than low-density particles. Similarly, feed pellets with high density and rumen fluid stability may provide increased rumen escape. Since conventional pelleting provides limited ability to control density and fluid stability, it was hypothesized that feed pellets with high density and fluid stability produced using extrusion technique will increase the rumen escape and improve utilization of starch and protein. Moreover, it was hypothesized that low-density (floating) extruded pellets with high fluid stability will provide better synchronization between nutrient demand and release in the rumen. Therefore, either through increased rumen escape or slow degradation of starch, extruded pellet types will benefit the rumen environment more than conventional pellets.

The research presented herein was conducted in three experiments. Firstly, if extruder processing could be used to produce feed pellets with physical properties (like density and fluid stability) targeted to affect the probability of rumen escape using *in vitro* techniques was studied (Paper-I). Then, the effects of density and fluid stability of feed pellets on rumen digestion behavior of starch and protein were studied by measuring digestibility, the postprandial duodenal appearance of starch and protein, and the postprandial rumen fermentation patterns in dairy cows using *in situ* and *in vivo* methods (Paper- II and III).

In Paper-I, barley, maize, soybean meal (SBM), and two mixtures containing barley + SBM (50:50) and maize + SBM (50:50) were used as feed material. The processing conditions used were two settings in a hammer mill (feed materials ground with either 2 mm or 6 mm screen size) and four extrusion settings (screw rotation speed either 210

rpm or 300 rpm, and application of cooling or not in the last section of the extruder barrel) in a twin-screw extruder. This study revealed that density and fluid stability of feed pellets from pure cereal (starch-rich) grains could be easily targeted by manipulation of screw speed and temperature in the last section of the extruder barrel, whereas feeds containing a high proportion of protein ingredients will require other processing settings.

In Paper-II, three pure barley extruded treatments having pellets of either low-, medium-, or high-density were used in a 3×3 Latin square design with three cannulated lactating Danish Holstein cows. In Paper-III, four treatments containing 70% barley and 30% SBM (as-is basis) were used in a 4×4 Latin square design with four cannulated lactating Norwegian Red cows. One treatment (control) was pelleted by conventional pelleting after expander processing, whereas the other treatments were extruded using three distinct settings giving pellets with either low-, medium-, or high-density. Conventional pellets had high-density but markedly lower fluid stability compared with extruded pellets.

Both experiments (Paper- II and III) demonstrated that high-density extruded pellets have a lower rumen degradation rate and greater rumen escape of starch, and thus lower rumen starch digestion (RSD) than other density pellets. However, despite having lower fluid stability and a higher starch degradation rate, the RSD of conventional pellets did not differ from the high-density extruded pellets. Although similar mean duodenal appearance, conventional pellets had a more rapid rumen outflow of starch after entering the rumen than high-density extruded pellets. About 98% of starch intake was digested up to distal ilium with all pellet types, indicating high small intestinal digestibility of starch. Consequently, the total tract digestibility of starch was more than 98% with all pellet types. Except for high propionate concentration in the dorsal rumen for low-density extruded pellets (Paper-III), no clear patterns for rumen fermentation variables were observed with respect to the physical properties of pellets. However, the acetate:propionate ratio was lower for low-density than high-density extruded pellets (Paper-II). Moreover, diurnal rumen pH was lower for extruded pellets than conventional pellets (Paper-III). In contrast to starch, no clear pattern of rumen digestion of protein with respect to the physical properties of feed pellets was observed. However, the duodenal flow of crude protein was higher for extruded pellets, especially low-density pellets, than for conventional pellets. Total tract NDF digestibility remained unaffected among treatments, but ruminal NDF digestibility was lower with extruded pellets than conventional pellets.

Overall, it can be concluded that the dynamics of rumen digestion of concentrate feeds can be manipulated by the physical properties of pellets, where density appeared to be the main property determining patterns of rumen digestion. Moreover, no evidence was found that extruded pellets are more beneficial for the rumen environment than conventional pellets. The findings in this thesis need further investigations.

Sammendrag

For å møte det høye kravet til innhold av energi og aminosyrer i rasjonen blir høytytende melkekyr tildelt kraftfôr med høyt innhold av stivelse og protein. God omsetning i vom er en hovedutfordring for best mulig utnyttelse av dette kvalitetsfôret. I Norge er lokalt produsert bygg, havre og hvete viktige energikilder til melkekyr. Rask fermenteringshastighet av stivelse i disse kornsortene er forbundet med økt risiko for sur vom, og derigjennom redusert energiutnytting. Tilsvarende kan rask nedbrytning i vom gi redusert utnytting av protein. Fordøyelse av stivelse og protein i tarm er assosiert med god utnytting av energi og protein. En forskyvning av fordøyelsen av stivelse og protein fra vom til tynntarm vil derfor gi økt fôrutnytting.

Tradisjonelt er fordøyelse i vom styrt gjennom valg av råvarer, eller påvirkning av nedbrytningshastighet ved behandling. Fordøyelse av næringsstoffer i vom er imidlertid et samspill mellom nedbrytning og passasje. Passasjehastighet er derfor en alternativ strategi for å styre fordøyelse i vom. Sammenlignet med nedbrytningshastighet er imidlertid passasjehastighet lite studert. Den viktigste faktoren som styrer passasje av fôrpartikler fra vom er egenvekt. Partikler med høy egenvekt har høyere passasje enn partikler med lav egenvekt. Tilsvarende kan pelletert fôr med høy egenvekt og høy stabilitet i væske gi økt passasje fra vom. Konvensjonell pelletering gir begrenset mulighet til å kontrollere egenvekt og væskestabilitet. Det ble antatt at pellets med høy egenvekt og væskestabilitet produsert ved bruk av ekstruderingsteknikk vil øke passasjen fra vom og dermed forbedre utnyttelsen av stivelse og protein. Videre ble det antatt at ekstruderte pellets med lav egenvekt (flytende) og høy væskestabilitet vil gi bedre synkronisering mellom tilgang og behov av næringsstoff i vom og at ekstrudert pellets derfor vil være mer fordelaktig for omsetningen i vom enn konvensjonell pellets, enten gjennom økt passasje, eller seinere nedbrytning av stivelse.

Denne avhandlingen bygger på tre forsøk. I det første forsøket ble in vitro metoder brukt til å undersøke om ekstrudering kunne brukes til å produsere pelletert fôr med fysiske egenskaper (som egenvekt og væskestabilitet) rettet mot økt sannsynligheten for passasje fra vom (artikkel I). Pelletert fôr med ulik egenvekt og væskestabilitet ble deretter undersøkt in situ og in vivo ved å måle fordøyelighet i vom og tarm, passasje av stivelse og protein til duodenum og gjæringsmønster i vom hos melkekyr (artikkel II og III).

I artikkel I ble bygg, mais, soyamel (SBM) og to blandinger (våt vekt basis) bestående av bygg + SBM (50:50) og mais + SBM (50:50) undersøkt. Behandlingene som ble benyttet var hammermaling på 2 og 6 mm sold og fire ekstruderingsinnstillinger (skruehastighet enten 210 eller 300 rpm med og uten kjøling i siste seksjonen av ekstruderen) i en dobbeltskrue ekstruder. Forsøket viste at egenvekt og væskestabilitet av pellets fra rein bygg eller mais (stivelsesrike) lett kan kontrolleres gjennom skruehastighet og

temperatur i siste seksjon av ekstruderen. Fôr med høy andel proteinråvarer (SBM og 50:50 blandingene) vil kreve andre behandlingsinnstillinger enn prøvd her.

I artikkel II ble reint bygg ekstrudert ved tre nivå for å gi pellets med lav, middels og høy egenvekt. Disse ble brukt i et 3 × 3 Latinsk kvadrat forsøk med tre fistulerte mjølkekyr av rasen Dansk Holstein. I artikkel III ble en blanding med 70% bygg og 30% SBM behandlet på fire nivå og brukt i et 4 × 4 Latinsk kvadrat forsøk med fire fistulerte mjølkekyr av rasen NRF. De fire nivåene var konvensjonell pelletering (kontroll) og ekstrudering med produksjon av pellets med lav, middels og høy egenvekt. Den pelleterte kontrollen hadde høy egenvekt, men markant lavere væskestabilitet enn de tre typene ekstrudert pellets.

Begge forsøkene (artikkel II og III) viste at ekstruderte pellets med høy egenvekt har lavere nedbrytning og høyere passasje, og dermed lavere fordøyelse av stivelse i vom, enn de andre typene pellets. Fordøyelse av stivelse i vom for konvensjonell pellets skilte seg imidlertid ikke fra ekstrudert pellets med høy egenvekt til tross for lavere væskestabilitet og høyere nedbrytningshastighet. Selv om duodenal flow var lik så var passasjen av stivelse fra vom raskere for konvensjonell pellets enn for ekstrudert pellets med høy egenvekt. For alle typer pellets så var ca. 98% av stivelsen fordøyd ved distale ileum, noe som indikerer høy fordøyelighet i tynntarm. Følgelig var fordøyelighet av stivelse totalt over 98% for alle typer pellets. Med unntak av høy konsentrasjon av propionsyre for ekstruderte pellets med lav tetthet i den ventrale delen av vomma (artikkel III), ble det ikke funnet noen klar sammenheng mellom fysisk kvalitet av pellets og gjæringsmønster i vom. Forholdet mellom eddiksyre og propionsyre var imidlertid lavere for pellets med lav egenvekt enn pellets med høy egenvekt (artikkel II). I tillegg var variasjon i pH i vom gjennom døgnet lavere for ekstruderte enn for konvensjonelle pellets (artikkel III). I motsetning til stivelse var det ingen tydelig sammenheng mellom fysiske egenskaper av pellets og fordøyelse av protein i vom. Duodenal flow av protein var imidlertid høyere for ekstruderte pellets, særlig for pellets med lav egenvekt, enn for konvensjonell pellets, noe som indikerer økt mikrobiell proteinproduksjon. Fordøyeligheten av NDF i vom og totalt var upåvirket av type pellets bortsett fra lavere fordøyelighet av NDF i vom for ekstruderte pellets sammenlignet med konvensjonell pellets.

Samlet kan det konkluderes med at fordøyelsesdynamikken av pelletert kraftfôr kan påvirkes gjennom fysiske egenskaper, og egenvekt syntes å være den viktigste egenskapen med hensyn på fordøyelsesmønster i vom. Forsøkene gir ikke grunnlag til å konkludere med at ekstruderte pellets er mer fordelaktig for vommiljøet enn konvensjonell pellets. Funnene i denne avhandlingen må undersøkes nærmere.

Abbreviations

AA	Amino acid(s)
BD	Bulk density
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
DP	Die pressure
EPD	Effective protein degradability
ESD	Effective starch degradability
FSG	Functional specific gravity
FSI	Fluid stability index
HDcon	High-density conventional pellets
HDext	High-density extruded pellets
HTST	High temperature short time
ISD	Intestinal (post-rumen) starch digestibility
LDext	Low-density extruded pellets
MCP	Microbial crude protein
MDext	Medium-density extruded pellets
MRT	Mean retention time
NDF	Neutral detergent fiber
N	Nitrogen
NPN	Non-protein nitrogen
RE	Radial expansion
RES	Rumen escape starch
RSD	Ruminal starch digestion
RUP	Rumen undegraded protein
SBM	Soybean meal
SD	Specific density
SISD	Small intestinal starch digestion
SV	Sinking velocity
VFA	Volatile fatty acid(s)
WSC	Water soluble carbohydrates

1 Introduction

1.1 General introduction

High-producing dairy cows have high demands for digested nutrients to meet their requirements for energy and amino acids (AA). Given that cows can eat a certain amount of feed daily, the requirements cannot be met solely by forages. Thus, animals are fed compound feeds in increasing quantities to provide sufficient levels of energy and AA. These compound feeds contain high amounts of starch and protein ingredients. In Norway, starch is provided mainly through domestically grown cereals where barley, oats, and wheat dominate. For protein, as in the rest of Europe, the ingredients are highly dependent upon import. In Norway, about 96% of protein ingredients used in livestock feeds are imported with soybean meal (SBM) and rapeseed meal (RSM) dominating (Landbruksdirektoratet, 2020).

Starch in barley, oats, and wheat is rapidly fermented in the rumen (Nocek and Tamminga, 1991; Larsen et al., 2009), which may restrict their use. Fed in too high amounts, these will negatively affect the rumen environment and reduce microbial efficiency, thereby protein efficiency and, in severe cases, both protein and energy efficiency (Owens et al., 1998). Moreover, the feed qualities needed often are costly, making the feed expensive. Thus, finding feed ingredients allowing an efficient feed utilization at an acceptable cost is important, and the feed industry urges for alternatives, either in the form of new feed ingredients or improved nutritive quality of existing feed ingredients. In the current thesis, improved energy and protein utilization in feed ingredients to ruminants through feed processing is focused.

In ruminants, efficient feed utilization is a balance between the digestion of nutrients in the rumen and the small intestine. The energetic efficiency of starch is higher when digested in the small intestine compared to degradation and fermentation in the rumen (Owens et al., 1986; Reynolds, 2006; Brake and Swanson, 2018). In addition, digestion of dietary protein in the small intestines is associated with less losses than protein fermentation in the rumen (Dijkstra et al., 2013). Moreover, shifting parts of readily digestible starch from the rumen to the small intestine probably will reduce the risk of feed-related health problems like rumen acidosis (Krause and Oetzel, 2006). Thus, in high-yielding cows, shifting a part of starch and protein digestion from the rumen to the small intestine will improve the utilization of compound feeds. It will also lead to a rumen environment better suited for the digestion of forages, which in ruminants are the primary locally produced feed resources. However, increased ruminal starch digestion (RSD) may yield a high amount of microbial protein if release of energy and nitrogen is properly synchronized. With respect to rumen escape starch (RES), the capacity for digestion in small intestine is discussed (Owens et al., 1986; Huntington et al., 2006), and rumen and

intestinal digestion vary considerably among starch sources and feed processing techniques (Larsen et al., 2009). RES not digested in the small intestine is not utilized. Thus, efficient feed utilization in ruminants is affected by several interacting factors.

Rumen digestion is a result of the concurrent rate of degradation and rate of passage. Manipulating the relation between these two concomitant processes will alter the site of nutrient digestion. This can be done by feed processing methodology. In ordinary feed production, the processing is usually restricted to grinding on hammer mill and agglomeration by conventional steam pelleting or expander pelleting. In these processes, the rate of rumen digestion can be altered through the choice of feed ingredient (Offner et al., 2003; Moharrery et al., 2014), and, especially for expander pelleting, the process settings (Prestløkken, 1999). However, this strategy target only the rate of rumen degradation, which may not be perfect for all ingredients or nutrients within an ingredient. As an example, expander treatment of barley may decrease the rate of rumen digestion and thereby increase rumen escape of protein (Prestløkken and Harstad, 2001), but results in higher (91%) RSD (Prestløkken and Harstad, 2001; Tothi et al., 2003).

In contrast to the digestion rate, manipulating passage rate through processing has not been intensively studied. A high passage rate, especially if combined with a lower degradation rate, will increase rumen escape. For passage rate, functional specific density and particle size are the most critical factors (Lechner-Doll et al., 1991; Offer and Dixon, 2000; Dufreneix et al., 2019). In this regard, a high-density particle has a higher rumen passage than a low-density particle, and a similar case can be with feed pellets. However, the relations between specific weight, particle size, rumen microbes, and forage digesta particles are complex. In short, a low-density floating pellet and a very high-density sinking pellet both will have a reduced probability of rumen escape (desBordes and Welch, 1984), whereas an optimum high-density feed pellet may sink into the reticulum and increase the probability of rumen escape. However, a slowly degradable floating pellet with a low likelihood for rumen escape may provide an optimal balance between nutrient demand and nutrient release, thereby improving synchronization and microbial synthesis.

Conventionally pelleted feeds for ruminants typically have high density and low water stability (Larsen and Raun, 2018). These pellets probably will disintegrate rapidly in the rumen, most likely losing their structure and thus physical properties. Cooking extrusion is a versatile processing method being frequently used in the fish feed industry. Extrusion is used to produce feed pellets with high water stability (Welker et al., 2018), and the density of pellets can easily be adjusted to control sinking velocity in water (Sørensen, 2012). Manipulating passage and degradation properties of feed pellets for ruminants through targeted feed processing using the extruder technology is not studied earlier, except for Larsen et al. (2019). The present work aims to gain knowledge about the different physical properties of extruded feed pellets in relation to their digestion

behavior in the rumen and small intestine. Furthermore, as most tests to describe the physical properties of feed pellets like water stability and sinking velocity have been adapted to the needs in fish feeding systems and since the rumen differs from the sea, an additional important part of the work is to adapt these methods to the rumen environment.

The efficient utilization of nutrients in ruminants is complex due to a unique digestive system, where the rumen presents the main challenge. A detailed understanding of ruminal degradation and passage is needed. Thus, at first, a short overview of the ruminant's digestive system will be presented. Thereafter, the dynamics of nutrient digestion in the rumen with a special emphasis on factors affecting particulate matter passage will be discussed. Finally, possibilities of manipulating passage properties of concentrate diets from rumen through feed processing, and features of extruder cooking, in particular, will be described.

1.2 Digestive physiology of dairy cows

The digestive system of ruminants differs from monogastric animals as their stomach is composed of four compartments, including the fore-stomachs, which are the rumen, the reticulum, and the omasum, and the true stomach, the abomasum (Figure 1.1). The rumen is the largest compartment and is divided into several sacs by pillars. The reticulum acts as a checkpoint between rumen and omasum, allowing specific digesta particles to leave the rumen through the reticulo-omasal orifice. The reticulum is not entirely separated from the rumen, and together, they constitute the 'reticulo-rumen,' hereafter named the rumen. The rumen is a large anaerobic fermentation chamber containing a complex microbes ecosystem, including bacteria, protozoa, fungi, and archaea (McDonald et al., 2011). Feed material entering the rumen is subjected to fermentative digestion, which is the metabolic action of microbes (Cunningham and Klein, 2013). This process of digestion is aided by initial chewing and subsequent rumination. During comminution of feed, copious quantities of saliva containing bicarbonate and phosphate salts are produced, enabling a rumen pH of 5.5-6.8. Microbes attached to feed particles hydrolyze complex feed components into simple molecules by extracellular microbial enzymes. These molecules are then taken up by microbes and metabolized intracellularly for maintenance and growth. In return, the host animal is benefited by the supply of energy substrates (volatile fatty acids; VFA) and amino acids (microbial protein).

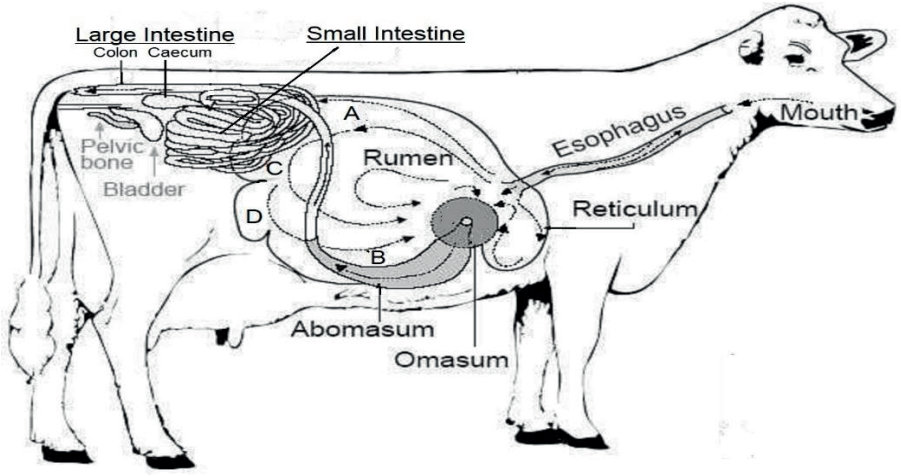


Figure 1.1 Schematic description of the digestive system of a dairy cow. A, dorsal sac; B, ventral sac; C, caudal-dorsal blind sac; D, caudal-ventral blind sac of rumen. Modified from Downing (2016).

Carbohydrates and proteins are the major components of dairy cow diets and constitute 60-70% and 15-20% of DM, respectively. Carbohydrates include structural (cellulose, hemicellulose, pectin) and nonstructural (starch, water-soluble carbohydrates

(WSC), fructans) carbohydrates, whereas crude proteins (CP; equal to N × 6.25) include true protein and non-protein nitrogen (NPN).

Cellulose and hemicellulose are present in the cell wall associated with lignin and collectively named neutral detergent fiber (NDF) after the analytical method (Mertens, 2002). Although pectin and β-glucans are also cell wall constituents, these are not recovered in the NDF fraction due to their solubility in boiling water (Van Soest, 1994) and are considered readily digestible in the rumen like nonstructural carbohydrates (Nocek and Tamminga, 1991). The end products of carbohydrates digestion in the rumen are VFA (mainly acetate, propionate, and butyrate), CO_2, and methane (CH_4). VFA are mostly (80-90%) absorbed into the blood through the rumen wall, whereas gases are lost by eructation.

The amino acids in dietary true protein are partly escaping rumen digestion and partly degraded in the rumen and converted to microbial proteins, ammonia, branched-chain fatty acids, and CO_2, whereas N of dietary NPN is added to the rumen ammonia pool (McDonald et al., 2011). Ammonia not used by microbes to synthesize protein is absorbed through the rumen wall and transported with the blood to the liver. Here it is converted to urea, which can be recycled to the rumen or excreted in the urine. Dietary protein and other feed components escaping ruminal digestion, together with microbes and fermentation products escaping the rumen, are subjected to digestion in the abomasum, small intestine, and large intestine (or hindgut) as in monogastric animals. Thus, in ruminants, digestion of feed components is the net result of microbial fermentation in the rumen, acidic and enzymatic digestion in the abomasum and the small intestine, and secondary fermentation in the hindgut.

1.2.1 Impact of the site of digestion on nutrient utilization

The different mechanisms of digestion throughout the gastrointestinal tract (GIT) affect the nature of absorbed substrates and thus the extent of nutrient losses and animal responses. For example, molar concentrations of acetate, propionate, and butyrate in rumen fluid, commonly referred to as the fermentation pattern or VFA profile, have important nutritional and metabolic consequences (France and Dijkstra, 2005; Cunningham and Klein, 2013). Through gluconeogenesis, propionate is the primary substrate of glucose needed for lactose synthesis, whereas acetate is an essential substrate for milk fat synthesis (Thomas and Martin, 1988). Thus, changes in VFA patterns are therefore related to milk production and composition. Several factors affect rumen fermentation patterns. Most important is the type of substrate (carbohydrate) with its availability and rate of degradation, encouraging the growth of specific bacterial species (Dijkstra, 1994). A starch-rich diet favors the growth of propionate-producing bacteria. In contrast, a fiber-rich diet enhances the proliferation of acetate-producing bacteria, although acetate is almost always the most abundant VFA (France and Dijkstra, 2005).

Microbial digestion of cell-wall carbohydrates is the most crucial process in the rumen because the nutrients in these compounds would otherwise be unavailable for the host. Although intestinal digestion of protein is preferred, the site of digestion is still under investigation for starch due to equivocal production responses. Several review articles have been published about starch (Owens et al., 1986; Nocek and Tamminga, 1991; Huntington, 1997; Huntington et al., 2006; Reynolds, 2006) and N compounds (Satter, 1986; Clark et al., 1992; Firkins, 1996; Calsamiglia et al., 2010; Dijkstra et al., 2013), discussing their relative importance of digestion and factors influencing it in the rumen or the small intestine.

Fermentative digestion of starch is metabolically less efficient than enzymatic digestion as a significant amount of energy is lost as gases and heat of fermentation. It has been estimated that only 50-70% of digestible energy is recovered as VFA when carbohydrates are digested in the rumen (France and Dijkstra, 2005; Goff, 2015). Using multiple regression, Owens et al. (1986) suggested that approximately 42% more energy was provided when starch was digested in the small intestine compared to the rumen in growing cattle. Reynolds et al. (2001) found in infusion studies that the energetic efficiency of starch is high when metabolized in the small intestine of dairy cows. Recently, Owens et al. (2016) concluded that energy efficiency could be increased by shifting the starch digestion site from the rumen to the small intestinal. Moreover, increased starch digestion in the small intestine has been suggested for enhancing milk protein production, perhaps by sparing amino acids from being used for gluconeogenesis in the liver (Nocek and Tamminga, 1991).

Apart from the energy efficiency considerations, increased RSD may negatively affect the rumen environment by lowering rumen pH (Owens et al., 1998; Khafipour et al., 2009). In particular, high producing dairy cows consuming high levels of rapidly fermentable starch are susceptible (Dijkstra et al., 2012). At low rumen pH, microbial activity decreases, leading to decreased fiber digestion, reduced efficiency in ruminal microbial protein synthesis, reduced dry matter intake (DMI) and decreased milk production (McCarthy et al., 1989; Allen, 2000). In severe cases, it may lead to acute or sub-acute rumen acidosis (SARA) associated with other health problems (Krause and Oetzel, 2006).

Concerning protein, extensive ruminal degradation may lead to excessive loss of dietary N as urea if ammonia is not captured by the microbes. In addition to decreasing N utilization, these losses may also contribute to environmental pollution through ammonia volatilization and nitrate leaching. In ruminant, the protein value of a feedstuff is dictated by the amount of AA, originating from microbial crude protein (MCP) and dietary protein escaping the rumen, absorbed in the small intestine. The synthesis of MCP depends upon the availability of N and energy derived from microbial fermentation. Under adequate supply of degradable N, MCP yield increases with increase RSD (Clark et al., 1992). Therefore, synchronizing ruminal degradation of starch and N is suggested as an adequate

strategy to increase MCP flow to the small intestine, thus increase N utilization. However, MCP cannot meet the demands of AA requirements in high-yielding cows.

A possible constraint for starch utilization in ruminants is the limited capacity of the small intestine to digest starch (Owens et al., 1986; Ørskov, 1986; Huntington, 1997). Similarly, the intestinal digestibility of dietary protein, escaping rumen degradation, can vary depending upon the source, processing, or antinutritive factors (Broderick et al., 1991; Calsamiglia et al., 2010). Moreover, potential effects of the site of digestion on productive responses are linked to responses in DMI (Reynolds, 2006), e.g., increased RSD can be beneficial if not related to metabolic disorders and reduction in DMI. Thus, efficient utilization of starch and protein in ruminants is a complex balance between rumen digestion and small intestinal digestion. Nevertheless, as yield increases, dairy cows will require an increasing part of dietary starch and protein that escape rumen digestion and subsequently digested in the small intestine to meet their energy and AA demands (Broderick, 2006).

1.2.2 Digestion of starch in the small intestine

Small intestinal starch digestion (SISD) begins in the duodenum, where pancreatic α-amylase hydrolyzes starch into disaccharides and oligosaccharides (i.e., maltotriose and branched limit dextrins) (Huntington, 1997; Harmon et al., 2004). These molecules are degraded into glucose by the brush border α-glucosidases (such as maltase, lactase, trehalase, glucoamylase) (Nozière et al., 2010), and glucose is absorbed across the brush border membrane mainly through the energy-dependent sodium-glucose transporter 1 (SGLT1) route. However, the energy-independent routes of glucose transporter 2 and glucose transporter 5, or paracellular absorption, can also be necessary at high luminal glucose concentrations (Brake and Swanson, 2018).

As stated, SISD in ruminant probably is limited, and 47 to 88% of starch entering the duodenum is reported digested in the small intestine depending upon the type of grain and processing (Owens et al., 1986). However, no plateau or upper limit in the quantity of starch digested post ruminally has been observed (Owens et al., 2016). A number of studies considering post-rumen digestion of starch either as a one-compartment (Nocek and Tamminga, 1991; Huntington, 1997; Patton et al., 2012) or differentiating into the small and the large intestine (Larsen et al., 2009; Moharrery et al., 2014), revealed a positive correlation between RSD and intestinal starch digestion (ISD). Larsen et al. (2009) suggested that the ingredient's intrinsic properties (e. g., particle size, degree of starch gelatinization), which influence RSD, also affect SISD similarly. Thus, rapidly degrading starch, such as barley and wheat, have higher intestinal digestibility than slowly degrading starch, such as corn and sorghum (Offner and Sauvant, 2004).

The capacity of the small intestine to digest starch has received considerable attention from many researchers (Owens et al., 1986; Ørskov, 1986; Huntington, 1997;

Mills et al., 1999b; Harmon et al., 2004; Harmon and Taylor, 2005; Huntington et al., 2006; Harmon, 2009). However, physiological factors explaining the limited capacity of SISD in ruminants remain a biological enigma. Generally, factors limiting SISD are thought to include the physical structure of starch, deficient enzyme activities, inadequate time for digestion, and reduced glucose absorption capabilities. The last two factors seem to be less limiting to SISD due to upregulation of both processes and well-suited digestive physiology of ruminants to maximize the absorption of free glucose (Brake and Swanson, 2018). However, ruminants seem to be deficient in the neuroendocrine control of pancreatic secretion to increased dietary starch due to their continuous flow of digesta into the small intestine. This has led to speculation that pancreatic α-amylase is the main limiting factor to SISD (Huntington, 1997). Moreover, it has been shown that greater intestinal starch flow may result in a concurrent down-regulation of the pancreatic amylase activity (Harmon, 2009). In contrast, Kreikemeier and Harmon (1995), by infusing glucose, corn dextrins, or corn starch abomasally, suggested that inefficiency of brush border α-glucosidases limited SISD. Recently, Mills et al. (2017), by modeling SISD, concluded that SISD is not limited by a single factor but series of rate-limiting steps involved in a complex interplay of hydrolysis and transport processes along the small intestine. They further suggested that it is crucial to consider glucose uptake by the small intestine rather than just starch disappearance for the actual energetic potential of SISD. Interestingly, increasing the supply of protein to the small intestine has been observed to increase SISD (Richards et al., 2002; Brake et al., 2014), probably through increased amylase capacity (Richards et al., 2003; Reynolds, 2006). However, the exact mechanisms, why increased small intestinal protein supply affects amylase capacity, is still unknown (Mills et al., 2017). A possible explanation is that increased luminal protein flow stimulates the secretion of cholecyctokinin (CCK) from enteroendocrine I cells in the small intestinal mucosa that in turn increases pancreatic secretions and thereby amount of amylase (Brake and Swanson, 2018). Thus, increasing the rumen escape of dietary protein together with starch will not only provide AA but may also improve SISD.

1.3 Dynamics of ruminal digestion

Rumen digestion is a dynamic sequence and synergy of the two concurrent processes, i.e., fractional rate of degradation (k_d) and fractional rate of passage (k_p). These processes are influenced by several factors like DMI, diet composition, physiological status of the animal, feed processing, and chemical alterations (e.g., fermentation, gelatinization) (Huntington, 1997; Giuberti et al., 2014). Ruminal digestion of a nutrient can be manipulated by changing the k_p/k_d ratio (Satter, 1986; van Staalen and Tamminga, 1990). Usually, this is done by affecting k_d through the choice of feed ingredients and/or various processing techniques. However, changing k_p can be an alternative approach to manipulate rumen digestion. An increased k_p, especially if combined with lower k_d, will

shift the digestion site from the rumen to the small intestine. However, before elaborating more on these two processes, it is essential to discuss the main challenge of measuring rumen digestion dynamics.

1.3.1 Methodologies to determine rumen digestion

In ruminants, ruminal degradation is measured using *in vitro, in situ* (or *in sacco*), and *in vivo* methods (Mertens, 2005), but each approach has its shortcomings. Using animals to measure the ruminal degradability of feeds *in vivo* is a reliable approach, but these methods are labor-intensive, time-consuming, and expensive. In comparison, *in vitro* and *in situ* methods are inexpensive which can produce estimates correlated to *in vivo* results (Gosselink et al., 2004). When applicable, k_d and extent of rumen digestion are most frequently measured with the *in situ* method. However, the method cannot be used on all feedstuffs and nutrients, and it cannot be used for measuring rumen passage. Passage kinetics can only be determined *in vivo* due to interactions between the diet and the animal. Usefulness and limitations of various methods used to study the rumen degradation of feeds have been evaluated in numerous studies (Nocek, 1988; Owens and Goetsch, 1988; Tamminga and Williams, 1998; Kitessa et al., 1999; Huhtanen and Sveinbjörnsson, 2006; Mohamed and Chaudhry, 2008; Velásquez et al., 2016): A short discussion of these methods will be presented here.

In vitro methods simulate *in vivo* digestion processes by employing suitable laboratory procedures and biological models. Several *in vitro* techniques have been developed. Usually, feed samples are incubated in a flask or tube containing rumen fluid (or feces) with a buffer (Lo´pez, 2005). Rumen degradation of a nutrient such as starch is then estimated either directly by measuring substrate disappearance after incubation for various time intervals or indirectly by measuring gas production. Some techniques involve cell-free enzyme medium instead of rumen fluid to estimate rumen digestibility. For proteins, some specific *in vitro* methods have been developed to estimate rumen degradability, including procedures estimating ammonia production after incubation in rumen fluid, N solubility, and using microbial markers (Lo´pez, 2005; Mohamed and Chaudhry, 2008). The advantages of *in vitro* techniques are many, including speed and flexibility, low cost, ability to assess individual feedstuff degradation and isolated from other interactions, small feed sample requirement, and ability to screen a large number of samples under similar experimental conditions. However, *in vitro* measurements may differ from results obtained *in vivo*. Starch degradabilities in the rumen estimated *in vitro* are usually lower than expected *in vivo* (Huhtanen and Sveinbjörnsson, 2006). This discrepancy is due to the limitations of *in vitro* methods which include but are not limited to: isolation from the rumen and other ingredients interactions, uncontrolled variations in the consistency of rumen fluid used, difficulty to simulate *in vivo* mechanisms governing particle size reduction, particle retention, nutrient addition, product accumulation and

removal, and limited time available for microbes during *in vitro* to adjust or adapt to a specific substrate (Owens and Basalan, 2016). Despite enormous efforts to address these limitations, no *in vitro* method has been generally accepted as a satisfactory alternative to *in situ* or *in vivo* methods.

In situ methods, like *in vitro*, mimic *in vivo* conditions, but feed samples are incubated directly in the rumen. Therefore, from a biological point of view, the *in situ* methods are more reliable than those of *in vitro* methods (Mohamed and Chaudhry, 2008). In this methodology, feed samples of known weights are sealed within porous nylon, polyester, or Dacron bags and placed in the rumen of a fistulated animal for varying time points (Hvelplund and Weisbjerg, 2000). After the required incubation time, the samples are removed, and subsequently, different feed components (such as DM, starch, protein) are determined in the washed residue. Despite being widely applied, this method is encumbered with errors that cause variations in results, both within and between laboratories. The major factors which affect the results include dimensions of the bag, the pore size of the bag, sample size, the particle size of the sample, time and numbers of incubation, and the rumen environment in which bags are incubated (Nocek, 1988; Nocek and Tamminga, 1991; Huntington and Givens, 1995; Mohamed and Chaudhry, 2008).

Although the methodology has been standardized to minimize errors (Åkerlind et al., 2011), more troublesome aspects of the *in situ* methods do exist. This method is usable for individual feeds, but not feeds that are soluble or have high particle loss (Offner et al., 2003). The fraction leaving the bag at 0 h of incubation is typically assumed to be soluble and completely degraded with an infinite or extremely high k_d which may not be true, especially for starch which is water-insoluble. In fact, this fraction is mainly comprised of small particles washed out from the bags (Tothi et al., 2003). Not correcting for particle loss may lead to an overestimation of ruminal degradation. By comparing *in vivo* and *in situ* measurements of starch digestion, Tothi et al. (2003) showed that the *in situ* method tended to overestimate *in vivo* RSD of rapidly degrading barley starch, but RSD of slowly degrading maize starch was tended to be underestimated. Related to the problem with particle loss, the major drawback of the *in situ* method is its assumption that the starch that disappeared from the bags is degraded. However, starch granules can be washed out from the bags without fermentation, either during incubation or during washing, thereby increasing apparent k_d (Huhtanen and Sveinbjörnsson, 2006). Therefore, *in situ* method is often criticized as method for measuring rumen degradation of starch. Similarly, protein feeds may also have particle loss however proteins can be soluble in water. To correct for particle loss, true soluble fraction can be determined as described by Hvelplund and Weisbjerg (2000), but true k_d in particle loss is unknown and is assumed to be equal to material remained in the bag. However, small washed-out particles may have different k_d than the material remaining in the bag (de Jonge et al., 2015). Another concern is different fermentation conditions inside and outside of the bag. A lower pH and different microbial

populations (both composition and concentration) have been observed inside the bags than outside the bags (Meyer and Mackie, 1986; Nozière and Michalet-Doreau, 1996; Krizsan et al., 2013). These may lead to lower activities inside the bags and underestimation of *in vivo* ruminal degradation by the *in situ* method. In addition to other factors, these different conditions inside and outside the bags could be due to the blockage of bag pores with fine particles (Vanzant et al., 1998). Microbial contamination of incubated feeds has been evidenced in many studies (Huntington and Givens, 1995; Nozière and Michalet-Doreau, 1996); however, it is usually not measured. Higher contamination of incubated feeds with rumen microbes will affect rumen degradation results, leading to underestimating DM and protein degradability. This problem is critical to consider when using high fiber feeds with low protein. Another limitation of *in situ* method, also *in vitro*, is that these methods ignore the impact of passage on extent of digestion. In order to simulate passage of potentially digestible fraction to calculate effective degradability (ED), a fixed value of k_p is used assuming first order kinetics. Since ruminal passage (even of starch) is not uniform (Tothi et al., 2003), it complicates kinetic simulations.

Despite limitations, both *in vitro* and *in situ* methods are very attractive approaches to rank and compare feeds or grain processing methods and screen feed samples for more detailed *in vivo* testing. Using multiple digestion measurements over time and computer models, k_d and potentially digestible fractions of nutrients can be calculated. These kinetic parameters of digestion are not only crucial in evaluating feedstuff degradability in the rumen but are also necessary for mechanistic nutrition models such as NorFor - The Nordic feed evaluation system (Volden, 2011).

In vivo determination, although the most logical approach, precludes measurement of ruminal degradation for individual feeds. Rumen degradation of a nutrient is typically determined by obtaining digesta samples from the duodenal or abomasal cannula and estimating digesta flow using markers (Johnson, 1966). To measure the digesta flow (expressed as g/d, mL/d, or g/h), the marker is provided constantly over a period of days, either infused directly into the rumen through rumen fistula or fed mixed with daily ration. Digesta samples are obtained once the steady-state conditions are assumed to have been achieved. Apart from flow rates, digesta flow from the rumen is usually presented as passage rate, measured as mean retention time (MRT) (Faichney, 2005). MRT is the time (hours or days) required for the passage of the averaged marked component, or an average particle spends in an organ. Ruminal MRT is the ratio between the amount of any component in the rumen digesta (pool) and the rumen outflow of that digesta component. Under steady-state conditions, k_p is the inverse of MRT and is expressed as h^{-1} or, when divided by 24, as d^{-1}. Pool size and k_p of an entity can be determined by administering markers as pulse dose attached to feed particles (Owens and Hanson, 1992), frequent

sampling of ruminal or fecal contents, and using various available kinetic models of flow (Ellis et al., 1994).

Using digesta flow rates, k_d and k_p of nutrients can be determined by the rumen evacuation technique (pool and flux method) (Stensig et al., 1998). In contrast to the marker technique, this technique allows the measurement of k_p of the potentially digestible fraction. However, both k_d and k_p are aggregated among different rumen compartments, which would hinder the determination of interactions of feeding combinations that alter mat consistency and buoyancy separate from the passage (Firkins et al., 1998). Moreover, rates cannot be determined for all nutrients (e.g., protein, which is disturbed by the microbes). For starch, it can be a suitable method as starch usually comes from concentrates. However, large diurnal variations in the rumen starch pool due to its rapid digestibility will violate steady-state conditions assumed for the rumen evacuation technique. This will require frequent rumen evacuations and careful selections of evacuation times to reduce diurnal variation in the rumen starch pool (Huhtanen and Sveinbjörnsson, 2006).

One of the main limitations of *in vivo* methods is the determination of accurate digesta flow from the rumen. A range of factors that can affect digesta flow measurements have been discussed (Titgemeyer, 1997; Firkins et al., 1998), including animals, cannula types, feed intake, markers, methods and schedules used for collection of digesta samples, replications, and calculations of data. Like other *in vivo* measurements, steady-state conditions are essential to calculate ruminal outflow. However, these conditions may never exist in practice, mainly due to infrequent feeding and improper mixing of marker with digesta (Owens and Hanson, 1992). Deviations from steady-state cause marker concentration to vary. In addition, simple T-shaped cannulas are known to provide an unrepresentative proportion of fluid and particulate matter in the digesta samples relative to true digesta. Unrepresentative sampling is the major problem increasing the estimated flow bias (Titgemeyer, 1997), especially when diets are high in grains. Even the reentrant cannulas which completely divert the digesta cannot solve the problem completely. A double-marker method has been proposed to correct the non-representative samples (Faichney, 1975), but it could not eliminate the problem (Huhtanen and Sveinbjörnsson, 2006). Titgemeyer (1997) suggested using markers for different phases like a rare-earth for particulate-phase, Co-EDTA for fluid-phase, and Cr_2O_3 for total digesta. Moreover, various sources of error inherent in the marker procedures such as migration, marker digestion and absorption, biosynthesis, the sensitivity of marker analysis, and erroneous kinetic assumptions (Owens and Hanson, 1992) further complicates the determination of flow rates. Limitations due to markers are less problematic when primary goal is to define differences among treatments. Yet, conducting nutrient digestion studies require careful consideration of experimental designs and procedures.

1.3.2 Rate of digestion

The rate of ruminal digestion primarily depends on the intrinsic properties of nutrient and feed ingredients. The k_d ranges from 300 to 700% h^{-1} in WSC (Weisbjerg et al., 1998) to 2 to 8% h^{-1} in NDF fractions (Nozière et al., 2010). Using *in situ* method, k_d of starch varies from 2.4 to 58% h^{-1}, giving a wide range in effective rumen starch degradability (ESD) among feedstuffs (Offner et al., 2003). Hvelplund et al. (2009) used maize, wheat, barley, oat, and peas treated in different ways both chemically and physically, giving 20 treatments in total, and found a k_d range of 8 to 78% h^{-1} for starch with rumen evacuation technique. Based on starch content and ESD, Offner et al. (2003) determined the probable amount of ruminally degraded and undegraded starch for the different feedstuff, as shown in Figure 1.2. Their findings correlate well with *in vivo* determinations where RSD is ranged from 355 g/kg starch intake for maize and sorghum to 940 g/kg starch intake for wheat and barley (Huntington, 1997; Mills et al., 1999a; Reynolds, 2006).

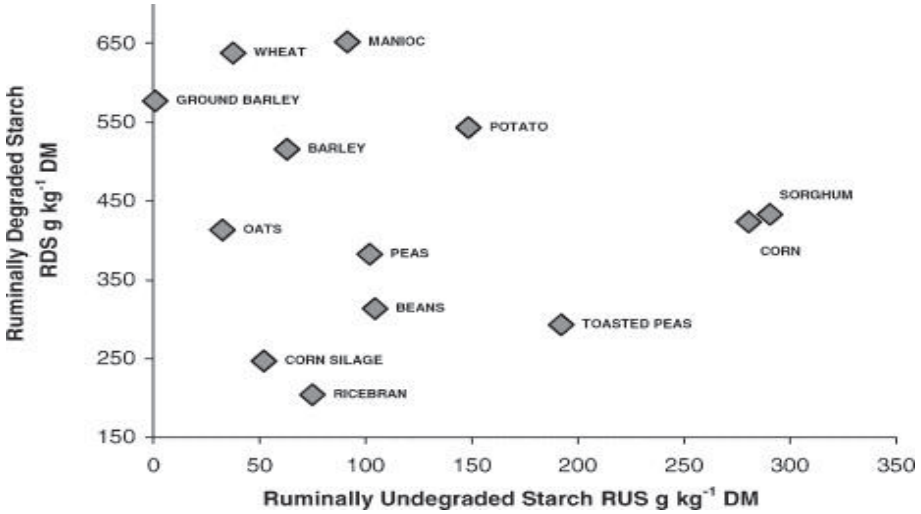


Figure 1.2 Contents of ruminally degraded and undegraded starch for different feedstuffs (Offner et al., 2003).

Interestingly, the rate of ruminal degradation of isolated cereal starches does not seem to differ from each other as determined *in vitro* (Cone and Wolters, 1990). Thus, apart from other factors, the rate of starch digestion is mainly dependent on inherent physicochemical properties of starch, including amylose:amylopectin ratio, granule morphology, degree of crystallinity, and most importantly, protein matrix surrounding starch granules (McAllister and Cheng, 1996; Svihus et al., 2005; Giuberti et al., 2014). In cereals such as barley and wheat, the protein matrix is easily hydrolyzed by the bacterial proteolytic enzymes, making starch more susceptible to bacterial amylase, whereas the

protein matrix in maize and sorghum is highly resistant to bacterial proteolytic enzymes (McAllister et al., 1993). Therefore, k_d and ESD of maize and sorghum are lower than for barley and wheat. The k_d of starch can also be affected by different processing techniques, especially heat treatment (Theurer, 1986; Offner et al., 2003), as discussed later.

Like starch, the rate of rumen digestion of dietary protein varies among feedstuffs. In concentrate ingredients, a range in k_d of protein from 1 to 22% h^{-1} is reported (van Staalen and Tamminga, 1990; Schwab et al., 2003), and thus, effective rumen protein degradability (EPD) varies among feedstuffs (Madsen and Hvelplund, 1985; Prestløkken, 1999; Ljøkjel et al., 2003). Inherent physicochemical properties of proteins like crosslinking (Satter, 1986), differences in proportional contents of rapidly (soluble albumins and globulins) and slowly (insoluble prolamins and glutelins) degradable proteins (Ljøkjel et al., 2003), and anti-nutritional components such as tannins affect EPD. The rate and extent of rumen degradation of protein in concentrates can also be altered through feed processing.

1.3.3 Rate of passage and factors affecting passage of digesta particles

The flow of digesta from the constantly mixed rumen pool is continuous; however, the passage of particles is not random. Newly ingested particles are selectively retained based on their physicochemical properties and animal factors (Lechner-Doll et al., 1991). Generally, particles with a larger size and high proportion of digestible material are retained longer in the rumen than small and indigestible particles. Thus, roughages are retained for a longer duration in the non-escapable pool in the rumen due to their large size and slow degradation. The k_p of forages ranges from 0.027 to 0.052 h^{-1} as calculated by Offner and Sauvant (2004) from a large database (n=316). Due to complex differential passage of fiber particles, it has received considerable research attention (Allen and Mertens, 1988; Mertens, 1993; Huhtanen et al., 2006; Krämer et al., 2013).

In contrast, concentrate particles are small and may pass faster out of the rumen than large forage particles. Therefore, the rumen degradation of starch and proteins in concentrate feeds is assumed to follow one compartmental model with first-order kinetics (Ørskov and McDonald, 1979). The k_p of concentrates starch is higher than forages, ranging from 0.030 to 0.078 h^1 (Offner and Sauvant, 2004). These calculated values are supported by the findings of Hvelplund et al. (2009), who observed a k_p range of 0.046 to 0.068 h^1 for starch using the rumen evacuation technique. However, Tothi et al. (2003) observed that the passage of undegraded starch out of the rumen increased at lower rates, subsequently peaking at 4-6 h post-feeding for barley and maize fed either as meal or expander pelleted. They further elucidated that k_p of starch was not constant over time for different rumen evacuations, indicating that starch passage does not follow the simple first-order kinetics. In contrast, Larsen et al. (2019) observed an exponential decline in

the flow of starch from the rumen for wheat and maize fed either conventionally pelleted or extruded pelleted. This shows that the passage of starch and thus concentrates out of the rumen can be equally complex as the passage of fiber particles.

Digesta flow from the rumen is a complicated process where k_p of particles is affected by several factors, including dietary, animal, and climatic (Lechner-Doll et al., 1991; Offer and Dixon, 2000; Faichney, 2005). Many studies have verified an increase in passage rate with the increase in feed intake, but the passage rate decreased as concentrate to forage ratio increased (Robinson et al., 1987; Colucci et al., 1990; Okine and Mathison, 1991; Dias et al., 2011). Moreover, the passage rate increases with the maturity of forages (Rinne et al., 1997). However, these effects of dietary changes on passage rate are not simple and determined by several interacting mechanisms by which rumen fill, particle comminution, and rumen's propulsive activities are regulated. Physical properties of feed particles, i.e., particle size and density, are the main determinants affecting the passage of digesta particles (Poncet, 1991). Prior to discussing these properties in detail, it is essential to explore particle flow dynamics in the rumen.

1.3.3.1 Particle dynamics in the rumen

Feed particles entering the rumen are separated into distinct layers according to their floatation-sedimentation velocities (Sutherland, 1988). The motility of rumen plays a major role in the movement of ingesta within and out of the rumen through the reticulo-omasal orifice. About 1-3 mixing contractions per min occur, which increase during eating and, for course fibrous feeds (Cunningham and Klein, 2013). These contractions make the particles circulate in two streams in the rumen (Wyburn, 1980), i.e., one in the dorsal sac and another in the ventral sac (Figure 1.3).

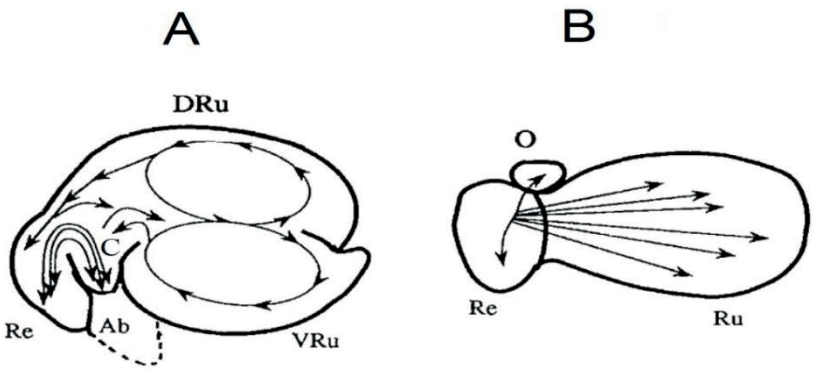


Figure 1.3 The patterns of digesta movement in the rumen in the horizontal (A) and vertical (B) planes. DRu; Dorsal rumen, VRu; Ventral rumen, Ab; Abomasum, Re; Reticulum, C; Cranial sac, O; Omasum, Ru; Rumen. (Adapted from Poncet (1991) after Waghorn and Reid (1977))

The newly ingested sufficiently dense particles may sink into the cranial sac, the reticulum, or the ventral rumen and will have a higher chance to bypass the rumen. Therefore, these particles constitute the 'escapable pool.' In contrast, light and buoyant particles are pushed into the dorsal sac, accumulating into fiber-mat floating on the liquid phase. They will have a low probability of rumen escape, constituting the 'inescapable pool' (Figure 1.4). Although particles are hydrated with rumen fluid, lighter particles' buoyancy may initially increase because of gas bubbles from microbial fermentation adhering to particles (Cunningham and Klein, 2013). However, as time passes, they start becoming denser and smaller due to an increase in hydration by rumen fluid and breakdown by mastication and microbes. As they move further caudally, they tend to get lower in the rumen and eventually enter the ventral rumen cycle. Ultimately, particles reach the ventral rumen wall, and from there, they can be pushed into the ventral cranial sac, while some less dense particles can be pushed back into the dorsal sac during ventral sac contractions. The contractions in the ventral cranial sac further separate the particles, and the smaller, highly dense particle are poured back into the reticulum. Once in the reticulum, these particles can be passed out through the reticulo-omasal orifice during the second phase of primary reticular contraction.

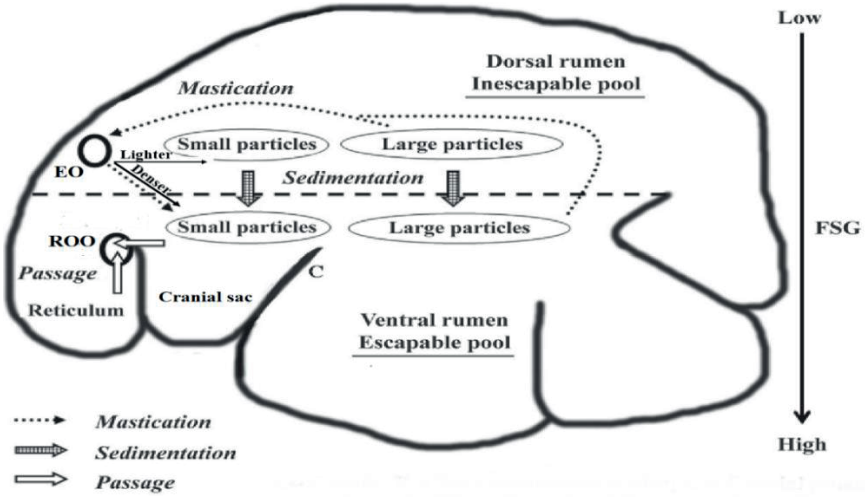


Figure 1.4 Graphical representation of particle dynamics in the rumen. EO, esophageal opening; ROO, Reticulo-omasal orifice; C, Cranial pillar; FSG, Functional specific gravity (corresponds to the density of particles, discussed below). Modified from Seo et al. (2009)

The effects of particles' physical characteristics on particle flow dynamics through the rumen have been extensively investigated using inert plastic particles or labeled indigestible plant cell walls (desBordes and Welch, 1984; Ehle and Stern, 1986; Murphy et al., 1989; Dufreneix et al., 2019). Studies with inert plastic particles can better elaborate the particle flow dynamics through the rumen compared to digesta particles for several reasons. Particles of forages and grains have different physical characteristics and

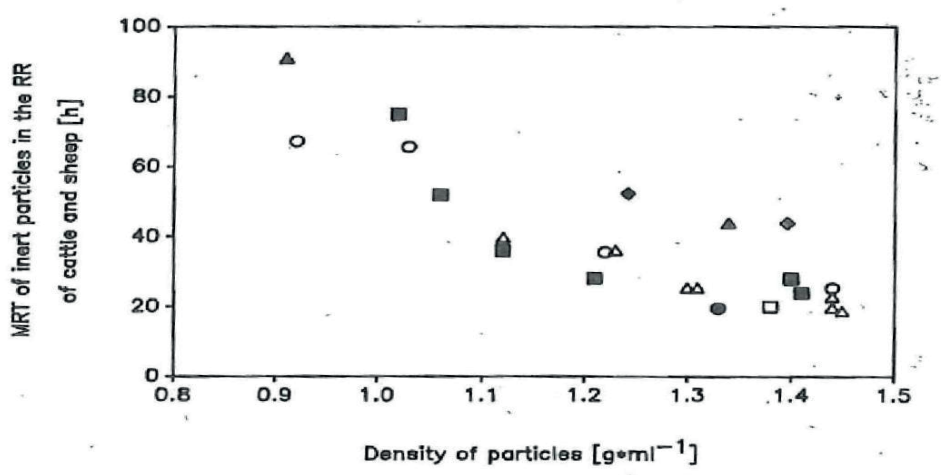
therefore exhibit a different representation of density-size relationships in various particle categories, which may contribute to anomalies (Kennedy, 2005). In contrast, the density and size of inert plastic particles remain homogeneous as these are not altered by hydration, bubble occlusion, or microbial breakdown. Although they can also be subjected to rumination, which may decrease their size (Kaske et al., 1992), their intrinsic physical properties, particularly for density, remain unchanged.

It is accepted that ruminal contractions discriminate particles with respect to size and density equally from moving out of the rumen. Sutherland (1988) found denser digesta particles in the reticulum compared to the dorsal and ventral rumen. Similarly, studies with plastic particles indicate that particle density is twice as important as particle size in determining the passage from the rumen. Kaske and Engelhardt (1990), using plastic particles with various densities and lengths, studied the contribution of size and density to MRT in the rumen. By regression analysis, they estimated that 59% of the total variation of MRT could be explained by particle density, whereas particle size determined 28% of the total variation of MRT. However, they also observed that particles with a length of 10 mm retained about 24 h longer than particles with a 1 mm length of the same density. In fact, the size and density of particles are physically dependent (Poncet, 1991).

1.3.3.2 Effects of particle density

The likelihood of particles outflow from the rumen is determined mainly by their density. Density is often described by the term functional specific gravity (FSG), which is the weight of a given volume relative to the same volume of water at the same temperature and pressure (Fuller, 2004); or simply, a ratio of the density of particle to the density of a fluid (e.g., water). However, in the rumen, particle density or specific gravity is affected by many factors, including structural components of particles, the fluid, internal gas components, and attached gas bubbles (Kennedy, 2005). These contributions from different factors make the measurement of FSG of digesta particles extremely difficult. Wattiaux et al. (1992b) used the terms; FSG and unit specific gravity (USG), defined as the specific gravity of the solid and gas fractions and the specific gravity of the solid, gas, and liquid fractions of digesta particles, respectively.

The density of digesta particles ranges typically from 0.8 g/mL to 1.5 g/mL (Evans et al., 1973). However, the initial density of fiber particles in the rumen can be as low as 0.6 g/mL (Hooper and Welch, 1985), whereas the initial density of concentrate particles can be up to 1.6 g/mL (Ramanzin et al., 1994). Lechner-Doll et al. (1991) demonstrated the influence of particle density on MRT in the rumen of cattle and sheep. They compiled the data from studies using either inert plastic particles or labeled indigestible plant cell walls and found a clear negative linear relationship between MRT in the rumen and particles' density (Figure 1.5). It shows that particles with densities between 1.3 to 1.5 g/mL had lower MRT in the rumen and would have higher passage rates from the rumen.



Sheep: △ Lindberg (1985), □ Katoh et al. (1988), ○ Kaske and Engelhardt (1990)

Cattle: ■ Campling and Freer (1962), ● Ehle et al. (1984), ◆ Ehle (1984), ▲ Ehle and Stern (1986)

Figure 1.5 Mean retention times (MRT) of small particles of different densities in the reticulorumen (RR) of cattle and sheep. (From Lechner-Doll et al., 1991)

The above relationship includes particles with density range generally suggested for digesta particles and therefore did not include particles with densities beyond 1.5 g/mL. desBordes and Welch (1984) used plastic particles of the same size (1x5 mm) with a range of densities (.90, .96, 1.17, 1.42, 1.77, and 2.15 g/mL) to study the effects of particle density on passage from the rumen in cows. They observed that passage rates of particles increased as the density of particles increased from 0.90 to 1.42 g/mL, but the particles with density 1.77 and 2.15 g/mL passed slowly. Besides, they observed that particles with a density below 1 g/mL were heavily ruminated, indicating that these particles were floating in the dorsal sac. However, Kaske et al. (1992) did not find any relationship between particle density and the probability of rumination.

The findings from desBordes and Welch (1984) gave a curvilinear relationship between particle density and MRT in reticulorumen. Similar findings were reported by Ehle and Stern (1986) and Murphy et al. (1989), who observed decreased passage of plastic particles with density 0.91 and 2.30 g/mL and 1.10 and 1.77 g/mL, respectively, compared to particles with density 1.34 g/mL. However, all these studies were conducted using low-producing animals (dry cows, heifers, steers, or sheep) and, therefore, low DMI. Since intake is proportional to particles' passage rate, high-yielding dairy cows may have different responses to particle density. Recently, Seyama et al. (2017) and Dufreneix et al. (2019) studied the effects of particle density on passage dynamics using inert plastic particles in lactating cows with an average DMI of 21.8 ± 1.75 kg/d. Seyama et al. (2017) found that particles with a density of 1.19 and 1.41 g/mL have a higher recovery rate in

feces after 120 h post-administration than 0.95 and 2.20 g/mL particles. Comparably, Dufreneix et al. (2019) observed shorter MRT in the rumen for 1.1 and 1.3 g/mL particles than for 0.9 and 1.5 g/mL particles. Hence, their results were consistent with the previous studies. Dufreneix et al. (2019) suggested that the density of ≤ 1.0 g/mL is too low to allow sedimentation of particles in the rumen, restricting the passage of particles out of the rumen. On the other hand, particles with very high density, i.e., ≥ 1.4 or 1.5 g/mL, easily sediment in the rumen compared to low-density particles but are not readily transported with liquids once they are in the ventral sections of the rumen and thus have a longer MRT in the rumen.

Feed particles are not inert like plastic particles, and their density change during time spent in the rumen. The FSG of feed particles is affected by two main factors, i.e., hydration and gas production. Hydration with saliva and rumen fluid increases the density of particles by replacing entrapped gas with liquid. The density of feed particles increases more rapidly in fresh rumen fluid *in vivo* than in autoclaved rumen fluid and water *in vitro* (Hooper and Welch, 1985). This greater increase in the density during *in vivo* was ascribed to microbial digestion and ruminal contractions that mix the digesta and increase hydration rate. In addition, density increase under hydration is dependent on feed material, particle size, physical cell structures (gas voids), and proportion of soluble and insoluble DM fractions (Hooper and Welch, 1985; Wattiaux et al., 1992a; Ramanzin et al., 1994; Bhatti and Firkins, 1995). For example, water holding capacity, and thus hydration, of particles increases with fibrous fractions compared to protein fractions (Ramanzin et al., 1994). In contrast to hydration, fermentative gases decrease the FSG of particles and make the particles more buoyant. By employing *in vitro* digestion, Wattiaux et al. (1992b) studied the change in FSG of particles due to gas production under microbial fermentation. They observed an increase in gas production and a decrease in FSG of particles between 3 and 9 h of incubation, but after that, an increase in FSG as the gas production decreased. However, Hooper and Welch (1985) did not observe this decrease in FSG during the first hours of microbial fermentation. Thus, the density of newly ingested feed particles probably first increases due to hydration and after that may decrease due to gas bubbles from microbial fermentation adhering to it. When digestible material depletes, density will increase again because microbial fermentation and gas production decreases.

1.3.3.3 Effects of particle size

In comparison with density, the effect of particle size on passage from the rumen has been investigated in numerous studies because of its relative ease of measurement. Particle size measurements are usually carried out by wet or dry sieving techniques, using screens of differing aperture and a sufficient sieving time (Kennedy, 2005). The size of particles leaving the rumen is commonly reflected by the particle size distribution of fecal contents, although a slight reduction in particle size may occur during passage through

the intestinal tract (post-rumen). Based on these measurements, the probability of a particle leaving the reticulorumen is inversely related to its size (Poppi et al., 1980; Weston and Cantle, 1984; Shaver et al., 1988; Kovács et al., 1997; Bayat et al., 2010). Particles larger than a certain size rarely leave the rumen; hence, the concept of "critical particle size" is often used where particles larger than this threshold size rarely leave the rumen. Critical particle size can be defined as particles retained on a screen with an aperture size between 1-2 mm (usually 1.18 mm is used) for sheep and cattle (Poppi et al., 1980; Lechner-Doll et al., 1991). However, estimation of particle size from sieving techniques is somewhat arbitrary as it is influenced by the length and shape of particles, the shape of the aperture, sieving time, agitation, and mass of particles applied to sieves (Poncet, 1991; Kennedy, 2005). In addition, these measurements predominately include undigested forage particles since concentrates particles usually are either reduced to very small size or fully digested before being excreted in feces. Very small particles can retain longer in the rumen than expected, which is attributed to the entrapment of particles in the fiber-mat, and this phenomenon is known as the "filter-bed effect" (Faichney, 1986). The studies where cows were fed whole grain diets revealed that a substantial amount of whole grain (larger than 4 mm) could appear in feces. Terada et al. (1987) found large amounts of undigested corn gains when cow feces were sieved with a 4.76 mm mesh screen. By investigating the distribution of undigested corn particles in Holstein steers' feces, Lee et al. (2002) found that particles, retaining on 4 mm and 8 mm sieve, were approximately 8-10% of feces dry matter. These findings suggest that the passage mechanisms of undigested grain particles could be different from forage particles. One possible explanation could be the interaction between particle size and density. Large forage particles typically have low density, whereas grain particles have a high density (Ramanzin et al., 1994). On the other hand, smaller particles have higher intrinsic density due to a higher surface area to volume ratio and poor gas entrapment (Offer and Dixon, 2000). Therefore, large forage particles first need to be reduced in size to attain optimum density for escape from the rumen.

Studies with inert plastic particles, having the same density, also revealed that particle's retention time in rumen decreases with a decrease in size (Campling and Freer, 1962; Ehle and Stern, 1986; Murphy et al., 1989; Kaske and Engelhardt, 1990; Kaske et al., 1992; Prigge et al., 1993; Clauss et al., 2011). These studies' results were consistent except that Ehle and Stern (1986) found a longer retention time for particles with a diameter of 3.2 mm than particles with a diameter of 6.4 mm. In agreement with this, recently, Seyama et al. (2017) found that plastic balls with a diameter of 6.35 and 7.95 mm pass more quickly through the rumen than plastic balls with a diameter of 3.97 mm. Interestingly, both studies used sphere-shaped particles. In a study conducted by Kaske et al. (1992), cylindrical particles with lengths of 1, 5, 10, and 20 mm but having the same diameter (0.75 mm) and density (1.03 g/mL) were used to investigate the relationship between

particle size and particle passage. To prevent the particles' interactions and sedimentation to the ventral rumen, a buffer was used by replacing ruminal contents, and CO_2 was bubbled continuously through spargers at the bottom of the rumen. Of the initially introduced particles, 32, 25, 13, and 2% of the 1, 5, 10, and 20 mm long particles, respectively, left the rumen within 4 hours. These results indicate that the outflow of particles from the rumen decreased with an increase in particle size. However, the outflow rate of 10 mm particles was 6.5 times higher than that of 20 mm particles, whereas the outflow rate of 1 mm particles was only 2.5 times that of 10 mm particles. All these findings with inert plastic particles indicate that, apart from the size, passage from rumen could be influenced by shape of particles and particles several times larger than the critical particle size, can leave the rumen in considerable amounts.

The particle size of feed particulate matter can be affected by chewing, microbial degradation, and ruminal contractions, all leading to particle size reduction. Chewing during eating and rumination are the two predominant means of comminution of large particles. However, ruminative mastication is more critical for the continued comminution of large particles than eating chewing (Kennedy, 1985; Ulyatt et al., 1986; McLeod and Minson, 1988). Both processes have different functions concerning particle size reduction. Chewing during eating prepares the feed for comfortable swallowing and microbial degradation by compromising the structural integrity of plant tissues. On the other hand, chewing during rumination facilitates particles' clearance by reducing the particle size of refractory material and the positioning of particles in the reticulum (Kennedy, 2005). Moreover, time spent chewing in each process is affected by the animal and diet characteristics. Compared to sheep, the rate of chewing during eating in cattle is slower and less effective in reducing particle size (Ulyatt et al., 1986), and the effect of rumination on grains is less than for forages (Kennedy, 2005). Studying rumination activity with plastic particles (desBordes and Welch, 1984; Murphy et al., 1989) revealed that large particles with low density were more ruminated than large particles with high density, while small particles with high density were practically not ruminated. Microbial degradation apparently has no direct effect on particle size reduction, but indirectly, it aids in size reduction by increasing the fragility of fibrous particles, thereby improving breakdown efficiency during rumination (McLeod and Minson, 1988). In contrast to forage particles, microbial detrition can play an important role in the breakdown of concentrate particles (Lechner-Doll et al., 1991).

1.4 Feed Processing and site of digestion

Feed processing includes the treatment (physical, thermal, chemical) of a feed before consumption by the animal. In ruminants, concentrate feedstuffs are processed basically to enhance their nutritive value by increasing digestibility across the whole digestive tract. However, processing may also alter the site of digestion. Several processing methods

have been established, ranging from physical to thermomechanical to chemical, and are selected according to needs and concerned animals. Processing methods and responses in site and extent of digestion have been reviewed extensively (Theurer, 1986; Huntington, 1997; Rowe et al., 1999; Firkins et al., 2001; Owens and Zinn, 2005; Owens and Soderlund, 2007). Feed processing typically involves damage of grain kernel and a reduction in particle size to increase the surface area exposed for microbial and enzymatic attack. The most common feed processing techniques for cattle include grinding, dry rolling, steamrolling, steam flaking, steam pelleting, and expander pelleting. Discussing all the processes and their effects on the digestion site is beyond the scope of this thesis.

In general, conventional processing techniques increase both ruminal and intestinal digestion of starch in much the same way (Rowe et al., 1999) through affecting k_d of starch. This is probably due to the concept as presented by Larsen et al. (2009) that similar factors (e.g., particle size and protein shielding) limit the extent of starch digestion at both sites. Therefore, a higher starch escaping the rumen for maize and sorghum may not be digested post-ruminally entirely and can be wasted in feces. Thus, slowly degradable grains need intensive processing to increase their utilization. On the other hand, increased particle size reduction and gelatinization due to feed processing may improve SISD but result in higher RSD. Gelatinization is a physicochemical process that starch undergoes when applying heat, moisture, and/or pressure to semi-crystalline native starch granules (Svihus et al., 2005; Tako et al., 2014). The intermolecular bonds of starch molecules are broken down, allowing the hydrogen bonding sites to engage more water resulting in an irreversible swelling of the granules, leaching out of linear amylose molecules from the amorphous regions, and loss of crystallinity and birefringence (Lund and Lorenz, 1984; Parker and Ring, 2001). The unfolding of amylopectin during gelatinization makes this highly branched molecule subjected to enzymatic attack from several positions (Tester et al., 2004), thus increasing the overall digestibility of starch.

In contrast to starch, the effect of several heat processing techniques on decreasing k_d, leading to reduce rumen degradability of proteins and thereby increasing ruminally undegradable protein (RUP), has been reported for many feedstuffs (Broderick and Craig, 1980; Broderick et al., 1991; Arieli et al., 1995; Lykos and Varga, 1995; Pires et al., 1997; Prestløkken, 1999; Prestløkken and Harstad, 2001; Ljøkjel et al., 2003). Heat processing changes protein nutritive value and digestion partly by reducing protein solubility and partly by blocking reactive sites for microbial proteolytic enzymes (Broderick and Craig, 1980) by altering proteins' molecular structures (Khan et al., 2015). This effect is commonly termed as protein denaturation. Thus, conventional processing techniques may improve feedstuff's protein value but appear to hold little potential for enhancing SISD while maintaining the RSD at low levels, especially for readily digestible grains.

As discussed in section 1.3 that the site of digestion can also be altered by manipulating k_p, where the density of feed particles plays a major role. Although

manipulating passage rate through physical properties of feed is scarcely studied, the concept of feed density is not new in ruminant nutrition. Flake density is often used to measure steam flaking intensity (Theurer et al., 1999), e.g., 309 g/L flake is more intensively processed than a 386 g/L flake. As with other conventional methods, an optimal flake density is used to improve overall starch utilization by increasing both ruminal and intestinal digestibility. Thus, steam flaking is usually restricted to slowly degradable grains. In the rumen, the initial density of concentrate particles usually ranges between 1.3 to 1.6 g/mL (Ramanzin et al., 1994) and thus slightly higher than optimal (1.2 to 1.3 g/mL) for increased passage as suggested by Dufreneix et al. (2019). These concentrate particles with such a high density may have less probability of escape, and if readily degradable, they may increase acidic conditions, particularly in the ventral rumen. Providing concentrate particles with optimal density for passage can give more escape and a better rumen environment. Alternatively, low-density particles slowly fermenting in the dorsal rumen can better synchronize between nutrient demand and release without hampering the rumen environment adversely. It may improve energy utilization and microbial protein synthesis. Thus, by manipulating both the passage and degradation properties of feed particles, the digestion kinetics of nutrients can be tailored towards more efficient utilization. The density of feed particles cannot be controlled easily during conventional feed processing. However, pelleting may have this potential.

1.4.1 Conventional pelleting

Due to several benefits such as less segregation of feed particles, increased nutrient availability, increased hygiene of feed, and ease on-farm allocation (Behnke, 1996), concentrate feedstuffs in the form of compound mixtures are often pelletized for cows in modern dairy production. The overall process includes milling, mixing, conditioning, and finally, pelleting. For pelleting, conventional pellet presses are typically used where rollers press the preconditioned compound meal through a steel die to form cylindrical pellets. In ordinary pelleting, feed mash is conditioned predominately by steam, and temperature is usually maintained between 75-80 °C. In expander pelleting, the feed material is conditioned in a special way before pelleting. Feed material, with the steam addition, is pushed through a barrel and is subjected to high temperature (can be up to 130 °C) and pressure for a short time by resisting the flow with a cone-shaped resister at the outlet gate of the expander. For this reason, the expander process is referred to as a high-temperature short-time (HTST) process. As feed mash leaves the expander, the sudden drop in pressure and water evaporation cause feed particles to explode, resulting in a greater rupture of starch granules and denaturation of protein molecules than in the ordinary conditioning process. Then expanded material is pelletized with a conventional pellet press.

In both processes, starch is partially gelatinized; however, the extent of gelatinization differs between both processes depending on moisture and heat input. In ordinary steam pelleting, about 10 to 20% starch is gelatinized, whereas about 22 to 35% starch is gelatinized in expander processing due to high moisture (up to 80 g water/kg feed material added), temperature (above 100 °C), and pressure (Svihus et al., 2005). Apart from effects on starch digestibility, gelatinization increases the physical quality of feed pellets (Wood, 1987). The gelatinized starch acts as the liquid bridge between particles forming the pellet. Therefore, expander pelleting enhances both the nutritional value and technical quality of compound feed compared with ordinary pelleting.

In short, pelleting is the mechanical agglomeration of small particles into larger particles under moisture, heat, and pressure (Rowe et al., 1999). By controlling the density of these particles (pellets), the rumen passage of compound feeds could be manipulated. However, pellets produced by conventional pelleting typically have high density, and the potential to control density is very less during this process. In addition, these pellets have low stability in water (Larsen and Raun, 2018) and, therefore, may quickly disintegrate in the ventral rumen. A disintegrating pellet will lose its physical integrity and density properties and may induce acidic conditions in the ventral rumen. Thus, the density and fluid stability of compound feed pellets are probably the key properties that could affect the probability of rumen escape of feed pellets. As mentioned earlier, the extrusion cooking technique is predominately being used in the fish feed industry to obtain compound feed pellets with high nutrient availability combined with specific physical functional properties of pellets suitable for allocation in water, e.g., high water stability (Misra et al., 2002; Welker et al., 2018) and density which can be easily adjusted to control the sinking velocity of pellets in water (Sørensen, 2012). Thus, this technique can be an excellent alternative to conventional pelleting in ruminant feed processing to achieve feed pellets with desired density and fluid stability.

1.4.2 Extrusion pelleting: An alternative to conventional pelleting in ruminant feed processing

Extrusion is one of the most versatile processing techniques frequently used to design feed (mainly fish feed) and food with a wide range of properties. It is a complex and complicated technological process. A comprehensive description of this process can be found elsewhere (Riaz, 2000; Guy, 2001; Riaz and Aldrich, 2007; Maskan and Altan, 2011): A brief description will be presented here. After milling and mixing, an extrusion process includes a bin/feeder, preconditioner, extrusion cooker, and die/knife assembly (Figure 1.6). Principally, the extruder is very similar to the expander but differs in the intensity of treatment and method of shaping the final product (Riaz and Aldrich, 2007). Moreover, extrusion allows more water addition than in expander processing.

In the extrusion process, the feed material is kneaded and pushed through the barrel by means of one or more screws of different configurations and eventually pressed and shaped through the die at the end of the barrel. During extrusion cooking, temperatures can be as high as 200 °C, but residence time is usually 15-20 seconds; therefore, this process is called HTST. The increase in temperature mostly happens in preconditioner where moisture is added both in gas (steam) and liquid phase, thereby making the temperature and moisture content of preconditioned feed mash usually in the range of 80-95 °C and 20-30%, respectively. Temperature rise in the extruder barrel is mostly from mechanical energy dissipated from friction and shear stress through the rotating screw(s). This energy system is termed specific mechanical energy (SME). The amount of mechanical energy added can be affected by altering the screw configuration and screw speed (Sørensen et al., 2010; Kraugerud et al., 2011). However, the temperature can also be adjusted in the extruder barrel by injecting steam/water directly into the material or heating/cooling the barrel. This energy system is commonly called specific thermal energy (STE). As the feed material passes through the extruder barrel, molecular transformations like starch gelatinization and protein denaturation occur. Subsequently, feed mesh is converted into a homogenous, viscoelastic melt of the meal due to mixing, heating, kneading, and shearing processes. The melt's flow through the extruder barrel is resisted by the die plate, thereby elevating pressure. The pressure difference between the inside of the extruder and the external environment causes partial evaporation of water at the exit point. As a result, the feed material is expanded and is cut off by a knife to form pellets. The operating conditions can be adjusted to control the expansion and, hence, the characteristics of the finished feed pellets.

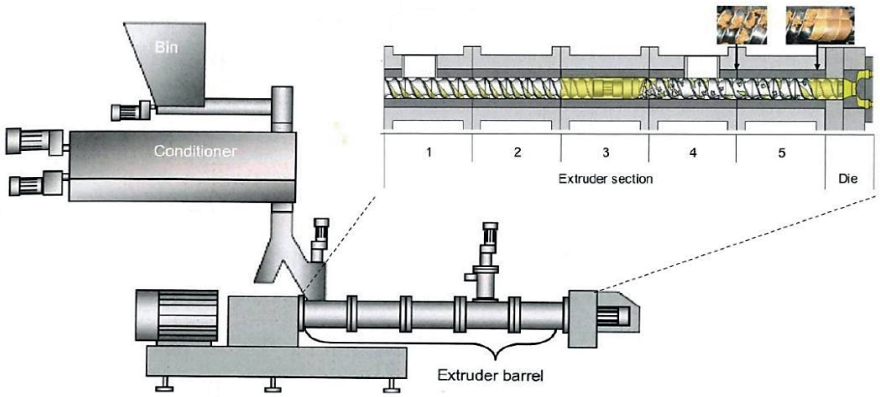


Figure 1.6 Schematic representation of extruder and its sections, including storage bin for meal and preconditioner. The yellow marking indicates the degree of fill of feed material and is exemplified by pictures at selected points. The open area in the extruder barrel in section 1 is the input from the preconditioner, and in section 4 is where a valve for venting or steam injection is located. Adapted from Kraugerud (2008)

Several types of extruders are used for processing animal feeds. Generally, extruders are divided into two major categories, i.e., single-screw and twin-screw. The extruder barrel is usually divided into sections. A twin-screw extruder (Intermeshed co-rotating; BCTG 62/20 D, Bülher AG, Uzwil, Switzerland) with five sections is shown in Figure 1.6. In twin-screw extruders, each screw comprises various elements giving the screw its configuration, which possesses different functions such as conveying, mixing, kneading, and cooking. These elements can be arranged in a variety of configurations as needed for specific applications. A typical screw configuration to produce fish feed is shown in Figure 1.6, where kneading elements are located in section 3, and cooking is mostly taking place in section 3 and section 5. Apart from the effects mentioned above, several beneficial functions occur during extrusion cooking in a short time, e.g., homogenization, texturization, binding of particles, forming/shaping, sterilization, and inactivation of antinutritional substances.

Extrusion cooking has key effects on the nutritive value and the physical quality of feeds regarding hardness, durability, sinking velocity, and water stability (Sørensen, 2012). If processed properly, the quality of extruded feeds is much better than the pelleted feeds. The extrusion process can be considered as a bioreactor where feed components, primarily starch, and protein, undergo complex and ill-defined processes involving, in many cases, irreversible changes in the physical and chemical structures. In general, changes in biopolymers that occur during extrusion cooking include cleavage, thermal degradation, loss of native conformation, binding, and fragment recombination (Steel et al., 2012). Due to high moisture and additional effects of shear forces, the extent of starch gelatinization (and melting) is greater during the extrusion process than in expander processing which can reach 100% depending upon ingredient, moisture, temperature, and shear (Camire et al., 1990; Svihus et al., 2005; Lundblad et al., 2011). Moreover, solid amylose-lipid complexes are formed during extrusion (Singh et al., 2007; Chen et al., 2011; Safaei and Yang, 2017). Similarly, the extent of protein denaturation is greater during extrusion, which reduces protein solubility, favors digestibility, and inactivates antinutritional factors such as antitrypsin, lectins, etc. In the extrusion, proteins undergo disruption and reorganization of disulfide bonds, non-specific hydrophobic and electrostatic interactions, cross-linking reactions, and possibly covalent bond formation (Arêas, 1992). In addition, the Maillard reaction may also occur during extrusion, where reducing sugars react with the free amine group of lysine or other AA (Camire et al., 1990). Hydrophobic protein matrixes, formed due to protein-protein interactions and cross-linking with proteins and other molecules, are enhanced upon cooling. On the other hand, gelatinized starch upon cooling returns to an insoluble, partially crystalline form composed of helices stabilized by hydrogen bonds (Englyst et al., 1992). All the above conditions enhance the physical quality (especially fluid stability) of extruded pellets. The most important parameters that affect the physical quality of extruded pellets are type of

material to be treated, particle size of the ingredients, preconditioning moisture and temperature levels, extruder configuration, screw speed, moisture added and temperature reached with in the extruder barrel, additional heating and cooling of each barrel section, die geometry, and residence time (Jansen, 1991; Lin et al., 1997; Suknark et al., 1999; Rolfe et al., 2001; Ainsworth et al., 2007; Chevanan et al., 2007; Altan et al., 2009; Miladinovic and Zimonja, 2010; Kraugerud et al., 2011; Sørensen et al., 2011; Fallahi et al., 2013). All these parameters make extrusion cooking processing the most flexible heat treatment.

The extrusion technique is rarely used in feed processing for ruminants and mostly to target rumen bypass proteins. As mentioned earlier that heat processing decreases rumen degradability of proteins, expander and extrusion processing has been reported to increase the proportion of RUP by decreasing k_d or solubility of the protein in cereal and legume grains during *in situ* (Prestløkken, 1999; Ljøkjel et al., 2003; Razzaghi et al., 2016) and this has been confirmed *in vivo* (Prestløkken and Harstad, 2001). Generally, heat processing, particularly with steam, increases starch digestion due to an increase in gelatinization (Svihus et al., 2005). Expander treatment of maize and sorghum has shown substantially increased ESD compared with untreated during *in situ* (Ljøkjel et al., 2003). However, expander and extrusion treatment of barley and wheat resulted in decreased k_d and ESD compared with untreated or ground barley meal (Offner et al., 2003). These findings are recently confirmed by Razzaghi et al. (2016), who reported increased ESD of extruded maize but decreased ESD of extruded wheat compared with untreated or conventionally pelleted treatments. Heat treatment at certain conditions favors the formation of more resistant protein matrix entrapping starch granules (Svihus et al., 2005), thus reducing starch digestion. This effect is particularly observed for readily digestible starch sources like barley, wheat. However, expander pelleting of barley did not support this notion and resulted in a higher (91%) RSD during *in vivo* studies (Prestløkken and Harstad, 2001; Tothi et al., 2003). Probably, due to the grinding action of conventional pellet press (Khan and Prestløkken, 2015), this protection of starch might have been lost during expander pelleting, thus resulting in higher RSD. Since the formation of pellet is different in extrusion, it can be postulated that starch protection can be preserved in extrusion pelleting. Thus, for ruminants, extrusion pelleting may have more potential to improve the utilization of starch and proteins in compound feeds by affecting both k_d and k_p simultaneously.

2 Aims, hypothesis, and objectives

The aim of the project, in which the present PhD thesis is part, was to improve energy and protein efficiency in dairy cows by altering the site of digestion of concentrate feedstuffs through targeted feed processing, thereby improving the profitability and sustainability of dairy farming. This PhD thesis aimed to study if that can be obtained through the extrusion cooking technique by producing feed pellets with the physical properties targeted to enhance rumen escape of starch and protein. The hypothesis was that feed pellets ranging in fluid stability and density would exhibit different rates of rumen degradation (k_d) and rate of rumen passage (k_p) of starch and protein, thereby improving energy and protein utilization in dairy cows.

Thus, this thesis's main objective was to investigate if extrusion technology can be used to produce feed pellets with physical properties targeted to alter ruminal digestion patterns in ruminants. This was tested through three research experiments where Experiment 1 (Paper-I) was solely for processing and *in vitro* testing of extruded pellets, and Experiment 2 and 3 (Paper- II and III) were *in vivo* trials to evaluate the effect of physical properties of feed pellets on digestion kinetics. On this basis, the following sub-objectives were established.

1) Study the production of extruded pellets and their behavior in rumen fluid in relation to the density and fluid stability by employing *in vitro* techniques.

2) Identify optimal density and fluid stability of pellets for increased rumen passage and investigate the critical processing factors required to achieve pellets with desired physical properties.

3) Study how density and fluid stability of feed pellets affect starch and protein utilization by measuring the rumen degradability *in situ* and digestibility, postprandial duodenal flow, and ruminal fermentation patterns *in vivo*.

4) Study the effects of altering the site of starch digestion on fiber digestion.

3 Materials and Methods

The detailed description of experimental procedures, chemical analyses, and statistical analyses used in three experiments are presented in their respective papers. A summary of applied methodologies within each experiment is described in this section.

3.1 Experiment 1 (Paper-I)

This experiment was designed to investigate if extruder processing could be used to produce feed pellets with physical properties targeted to affect the probability of rumen escape. The physical properties were evaluated using laboratory methods. The feed materials used were barley, maize, soybean meal (SBM), barley + SBM (B+SBM; 50:50), and maize + SBM (M+SBM; 50:50). The processing conditions used were two settings in a hammer mill (2 mm or 6 mm screen size) and four extrusion settings (screw rotation speed either 210 rpm or 300 rpm, and application of cooling or not in the last section of the extruder barrel) using 6 mm die size (revolver die; six number of dies) in a twin-screw extruder. The feed materials ground with 2 mm screen size in hammer mill were also extruded using 3 mm die size (revolver die; twelve number of dies), but these feeds were not included in Paper-I and are shown in supplementary results (section 5). All feeds were produced without replicates. The physical properties studied were radial expansion (RE), bulk density (BD), sinking velocity (SV), specific density (SD), and fluid stability index (FSI). Procedures for determining these physical properties are described in detail in Paper-I; however, as SV, SD, and FSI were modified, a brief description with figures will be presented here. SD was determined in quintuplicate by measuring the weight of five selected pellets and then the volume of the pellets by volumetric displacement method using 0.5 mm glass beads in a tapped density analyzer (AUTOTAP, Quantachrome Instruments, Boynton Beach, Florida, USA) (Figure 3.1). SV test was performed on 30 randomly selected pellets by measuring the time taken by a pellet to pass a distance of 220 mm in a transparent glass cylinder (310 mm high and 35 mm inner diameter), filled with rumen fluid of approximately 39 ℃ (Figure 3.2). The FSI of pellets was determined in triplicate by measuring the dry matter that remained in 2 mm mesh net ball-shaped baskets after incubation in rumen fluid at 39 ℃ for 30, 60, and 120 min (Figure 3.3). Pearson product-moment correlation procedure in SAS (2013) was used to check inter-relationships between variables. The MIXED procedure and repeated measurement statement of SAS (2013) were used to evaluate treatment effects on RE, BD, SV, SD, and FSI of pellets.

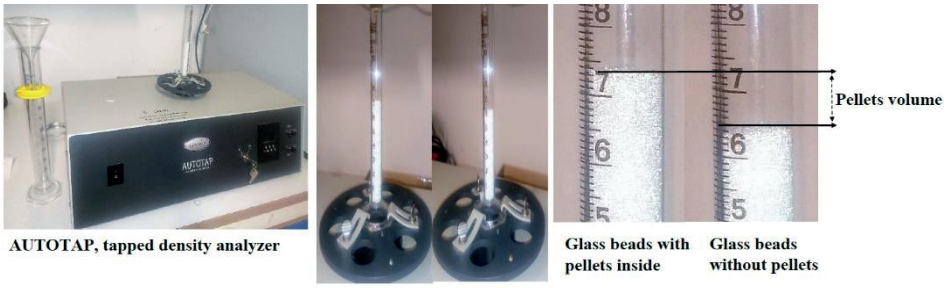


Figure 3.1 Demonstration for measuring the volume of feed pellets for specific density (SD) determination

Figure 3.2 Apparatus for measuring sinking velocity (SV) of feed pellets

Figure 3.3 Demonstration of fluid stability index (FSI) test up to incubation. After incubation, baskets were placed in an oven at 103 °C for 18 hours for drying

3.2 Experiment 2 (Paper-II)

In this experiment, the kinetics of starch utilization in dairy cows fed extruded pellets differing in physical functional properties was investigated by measuring starch digestibility, postprandial rumen fermentation patterns, and postprandial duodenal starch appearance. Additionally, the effects of starch digestion on neutral detergent fiber (NDF) digestibility and methane emission were studied. Pure barley was used during extrusion to produce three treatments with pellets of either low-, medium-, or high-density based on Paper-I findings. The three treatments were tested in a 3×3 Latin square experiment with 21-day periods having 11 days of adaptation and 10 days of sampling. The three lactating Danish Holstein cows fitted with ruminal, duodenal, and ileal cannulas were used. Due to problems with involuntary intake, all treatments were fed directly into the rumen through the rumen cannula in a way to simulate the entrance of pellets into the rumen by eating. After the allocation of experimental concentrate, cows were fed a basal diet low in starch. Titanium dioxide (TiO_2) was used as a digestibility marker by placing directly into the rumen at each feeding. Chromium ethylenediaminetetraacetic acid (Cr-EDTA) was continuously infused into the rumen to estimate postprandial duodenal digesta flow. Eight samples were collected on equal time intervals (9 hours) from duodenal digesta, ileal digesta, and feces (grab sample) to determine digestibility. For postprandial rumen fermentation patterns, four sample sets of rumen dorsal, medial, and ventral fluid were taken from each cow at 2, 4, 6, 8 h, whereas for postprandial duodenal starch appearance, samples of duodenal chyme were obtained from each cow at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16 h relative to morning feeding of the experimental concentrate at 07:00 h. Methane emission was continuously measured in the respiration chambers for the last two days in each period. Feed intake, nutrient digestibility, and methane emission data were statistically analyzed using the GLM procedure in SAS (2013). The postprandial and diurnal rumen fermentation variables and postprandial duodenal DM and starch flow were statistically analyzed using the MIXED procedure of SAS (2013) for repeated measurements. Detailed procedures are described in Paper-II.

3.3 Experiment 3 (Paper-III)

In this experiment, the effects of physical functional properties of feed pellets on nutrient digestion were investigated by measuring starch and protein digestibility, postprandial rumen fermentation patterns, and postprandial duodenal appearance of starch and protein in dairy cows fed a basal diet low in starch. Additionally, the effects of ruminal starch digestion (RSD) on neutral detergent fiber (NDF) digestibility were studied. Four treatment concentrate pellets were produced based on a compound concentrate meal containing 70% barley and 30% soybean meal (SBM; as-is basis). One treatment (control) was pelleted by conventional pellet press after expander processing and expressed as high-density conventional (HDcon) pellets, whereas the other

treatments were extruded using three distinct settings giving pellets with either high-density (HDext), medium-density (MDext), or low-density (LDext). The animal experiment was conducted in a 4×4 Latin square design with four treatments, cows, and periods. Each period consisted of 21 days, of which the first 11 days were used for adaption and the last 10 days were used for sampling. Four cows used were lactating Norwegian Red fitted with ruminal and duodenal cannulas: two cows also had ileal cannula. A dual-marker technique was applied using continuously infused chromium ethylenediaminetetraacetic acid (Cr-EDTA) and ytterbium acetate (Yb-acetate) as external markers to estimate the duodenal and ileal digesta flow. Over a period of three days, eight samples from duodenal and ileal digesta and total feces were collected for the determination of digestibility. For postprandial duodenal starch and protein appearance, fifteen sample sets of duodenal chyme were obtained from each cow at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 17 h whereas for postprandial rumen fermentation patterns, nine sample sets of rumen dorsal, medial and ventral fluid were taken from each cow at 0, 1, 2, 3, 4, 5, 6, 7, 8 h relative to morning feeding of the experimental concentrate at 07:00. To determine the diurnal rumen pH variations, pH was logged every 10th min for 24 hours. Rates of digestion (k_d) and passage (k_p) of starch were estimated using total rumen evacuation. Ruminal degradation characteristics of starch and protein for experimental treatments were also determined by *in situ* technique using nylon bags. Feed intake, nutrient digestibility, and rumen evacuation data were statistically analyzed with the MIXED procedure, whereas the *in situ* data was analyzed with the GLM procedure of SAS (2013). The postprandial and diurnal rumen fermentation variables and postprandial duodenal digesta flow were statistically analyzed using the MIXED procedure of SAS (2013) for repeated measurements. The detailed procedures for feed processing, feeding, sample collection, and analytical techniques are described in Paper-III.

4 Results

The current section includes the main findings of the conducted research, which are presented in three papers.

4.1 Paper I

Targeting nutrient utilization in ruminant diets through extruder processing: Production and measurement of physical properties of feed pellets

- The study revealed that maximum BD and SD for floating pellets was 469 g/L and SD 0.76 g/mL, and minimum BD and SD for fast sinking pellets was 570 g/L (SD 0.96 g/mL), respectively. In comparison, pellets with BD 502 g/L and SD 0.85 g/mL were slow sinking.
- The SD of pellets increased after immersion in rumen fluid from 0.006 g/mL to 0.31 g/mL, where this increase was higher for low-density pellets than high-density pellets.
- Both barley and maize feeds gave highly stable pellets with an average FSI of 89 ± 7%, whereas SBM feeds provided pellets with the lowest FSI (average 8 ± 3%). Mixture feeds were also less stable, giving an FSI of an average of 22 ± 11%.
- The type of feed material and cooling applied (or temperature) at the last section in the extruder barrel were the most critical processing parameters affecting the density and fluid stability of feed pellets, followed by screw speed and feed materials particle size.
- Overall, maize gave the highest RE and consequently lowest densities with more floating feeds. Maximum RE was achieved for the feeds ground at 2 mm screen size and extruded at 300 rpm without cooling in the last section.
- Cooling in the last section of the extruder barrel decreased RE in all feeds, giving high-density pellets, but this effect was higher for maize, giving pellets of the highest density with very fast SV.
- The highest density pellets were obtained for the feed ground at 2 mm screen size and extruded at 210 rpm with cooling applied at the last section in the extruder barrel.

4.2 Paper-II

Effects of the density of extruded pellets on starch digestion kinetics, rumen fermentation, fiber digestibility, and enteric methane production in dairy cows

- The digestibility of starch did not differ among the treatments in any segment of the digestive tract. The average digestibility of starch in the rumen, intestine, and total tract was 82 ± 4%, 95 ± 0.8%, and 99 ± 0.1%, respectively.

- About 98% of ingested starch was digested up to the distal ileum. Glucose contents in ileal digesta were low and did not differ among treatments.
- NDF digestibility and CH4 emission also remained unaffected by the treatments.
- High-density pellets showed a higher acetate:propionate ratio at all positions in the rumen and a higher postprandial duodenal starch appearance than low-density and medium-density pellets, indicating a lower RSD for high-density extruded pellets
- Thus, high-density extruded pellets had the highest rumen escape starch (RES) into the small intestine, where it was mostly digested and absorbed.

4.3 Paper-III

Effects of density and fluid stability of extruded barley-soybean meal pellets on digestion kinetics and rumen fermentation patterns in dairy cows

- Conventional pellets had markedly lower FSI compared with extruded pellets.
- RSD was lower for high-density pellets than other density pellets (87% *versus* 90%), but it did not differ between HDcon and HDext despite marked differences in FSI.
- Similarly, the postprandial duodenal appearance of starch was the highest for high-density pellets but with a more rapid appearance for HDcon than for HDext
- Nevertheless, k_p of starch determined by rumen evacuation did not differ among treatments.
- Although no significant differences, the k_d of starch determined *in situ* and *in vivo* was numerically lower for HDext than other treatments.
- Diurnal and postprandial dorsal and medial rumen pH patterns reached a lower nadir for extruded pellets than conventional pellets.
- Total VFA concentration in rumen fluid did not differ among treatments, but propionate concentration was the highest for LDext in the dorsal rumen.
- Total tract digestibility of starch was more than 99% for all treatments indicating high intestinal digestibility of starch with all pellet types. Two cows with ileal cannula indicate that more than 98% of ingested starch was digested up to the distal ileum.
- In contrast to starch, k_d of protein differed among treatments and was the lowest for LDext and the highest for HDext.
- The duodenal protein flow was higher for extruded pellets, especially for LDext, than conventional pellets.
- Ruminal digestibility of NDF was lower for extruded pellets than conventional pellets, but the total tract digestibility of NDF did not differ among treatments.

5 Supplementary results

As stated earlier, in Experiment 1, feeds produced by 2 mm screen size in hammer mill were also extruded with 3 mm die size. However, these feeds were not included in Paper-I because of practical reasons with statistical analysis and results interpretation. Due to their relative importance, those results are presented in the following tables and figures.

Table 5.1 Extrusion processing data of individual ingredients (barley, maize, and soybean meal (SBM)) and mixture (50:50, barley+soybean meal (B+SBM) and maize+soybean meal (M+SBM)) feeds produced with 2 mm screen size in hammer mill and 3 mm die size in the extruder.

	FM /Feed[1]	1	2	3	4	Overall[2]	
Screw speed (rpm)		210	210	300	300		
Cooling[3]		No	Yes	No	Yes	No	Yes
T3[4] (°C)	Barley	116	-	117	-	117 ± 0.5	-
	Maize	118	106	129	108	124 ± 6	107 ± 1
	SBM	118	108	121	107	120 ± 2	108 ± 0.5
	B+SBM	110	102	112	101	111 ± 1	102 ± 0.5
	M+SBM	110	104	110	103	110 ± 0	104 ± 0.5
T5[4] (°C)	Barley	117	-	120	-	119 ± 2	-
	Maize	114	82	127	89	121 ± 6	86 ± 4
	SBM	114	88	120	82	117 ± 3	85 ± 3
	B+SBM	107	81	113	82	110 ± 3	82 ± 0.5
	M+SBM	108	84	109	83	109 ± 0.5	84 ± 0.5
DP[5] (bar)	Barley	55	-	46	-	51 ± 4	-
	Maize	24	34	17	27	21 ± 4	31 ± 4
	SBM	42	47	36	44	39 ± 3	46 ± 2
	B+SBM	40	41	37	38	39 ± 2	40 ± 2
	M+SBM	34	34	27	35	31 ± 4	35 ± 0.5
Torque[6] (Nm)	Barley	387	-	315	-	351 ± 36	-
	Maize	357	375	319	329	338 ± 19	352 ± 23
	SBM	394	434	325	359	360 ± 34	397 ± 38
	B+SBM	322	310	275	283	299 ± 24	297 ± 14
	M+SBM	277	258	213	266	245 ± 32	262 ± 4
SME[7] (Wh/kg)	Barley	80	-	92	-	86 ± 6	-
	Maize	70	74	90	92	80 ± 10	83 ± 9
	SBM	75	87	90	103	83 ± 8	95 ± 8
	B+SBM	65	61	77.1	81	71 ± 6	71 ± 10
	M+SBM	56	52	61	77	59 ± 2	65 ± 12

[1] Feed material (FM) in the column and feed (treatment) number in the row.
[2] The values are averages (with standard deviations) of all the feeds for the respective feed material.
[3] Cooling at section last section in the extruder barrel.
[4] Temperatures measured by the sensor placed in the extruder barrel at each section (section 3; T3, section 5; T5).
[5] Die pressure
[6] Engine load, maximum torque is 435 Nm.
[7] Specific mechanical energy.

Table 5.2 Bulk density (BD), sinking velocity (SV), specific density (SD), and specific density in rumen fluid (SDrf)1 (with standard deviations) of individual ingredients (barley, maize, and soybean meal (SBM)) and mixture (50:50, barley+soybean meal (B+SBM) and maize+soybean meal (M+SBM)) feeds produced with 2 mm screen size in hammer mill and 3 mm die size in the extruder.

	FM /Feed[2]	1	2	3	4	Overall[3]	
Screw speed		210	210	300	300		
Cooling[4]		No	Yes	No	Yes	No	Yes
BD (g/L)	Barley	454 ± 3	-	396 ± 2	-	425 ± 29	-
	Maize	470 ± 3	746 ± 9	350 ± 3	538 ± 4	410 ± 60	642 ± 104
	SBM	626 ± 2	662 ± 2	617 ± 3	653 ± 2	622 ± 4	658 ± 4.5
	B+SBM	587 ± 2	661 ± 3	519 ± 5	620 ± 2	553 ± 34	641 ± 20
	M+SBM	658 ± 4	706 ± 2	627 ± 2	669 ± 7	643 ± 16	688 ± 18
SV (mm/sec)[5]	Barley	00	-	00	-	00	-
	Maize	00	94 ± 18 (80)	00	71 ± 30 (60)	00	83 ± 11 (70)
	SBM	107 ± 5	110 ± 1	105 ± 5	108 ± 4	106 ± 1	109 ± 1
	B+SBM	47 ± 11 (90)	95 ± 7	20 ± 11 (20)	69 ± 13	34 ± 14 (55)	82 ± 13
	M+SBM	83 ± 11	110 ± 1	71 ± 8	97 ± 6	77 ± 6	104 ± 6
SD (g/mL)	Barley	0.77 ± 0.01	-	0.64 ± 0.02	-	0.70 ± 0.06	-
	Maize	0.78 ± 0.02	1.16 ± 0.02	0.55 ± 0.04	0.84 ± 0.04	0.66 ± 0.12	1.05 ± 0.21
	SBM	0.96 ± 0.06	1.04 ± 0.07	1.08 ± 0.02	1.13 ± 0.05	1.02 ± 0.06	1.08 ± 0.04
	B+SBM	0.84 ± 0.05	0.93 ± 0.05	0.74 ± 0.05	0.90 ± 0.09	0.79 ± 0.05	0.88 ± 0.02
	M+SBM	1.04 ± 0.01	1.08 ± 0.03	1.00 ± 0.02	1.04 ± 0.12	1.04 ± 0.06	1.09 ± 0.09
SD_{rf} (g/mL)	Barley	0.90 ± 0.01	-	0.89 ± 0.01	-	0.89 ± 0.01	-
	Maize	0.97 ± 0.08	1.13 ± 0.01	0.80 ± 0.01	0.98 ± 0.02	0.89 ± 0.09	1.05 ± 0.08
	SBM[6]	-	-	-	-	-	-
	B+SBM	1.02 ± 0.03	1.06 ± 0.02	0.94 ± 0.02	1.04 ± 0.02	0.98 ± 0.04	1.05 ± 0.01
	M+SBM	1.04 ± 0.02	1.07 ± 0.06	1.02 ± 0.08	1.05 ± 0.03	1.03 ± 0.02	1.06 ± 0.01

[1] SD of pellets after soaking in rumen fluid at 39 °C for 20 min.

[2] Feed material (FM) in the column and feed (treatment) number in the row.

[3] The values are averages of all the feeds for the respective feed material.

[4] Cooling applied at the last section in the extruder barrel.

[5] Numbers in the parenthesis represent percentages of sinking pellets measured up to 20 min after dropping the pellet. (00) represent floating pellets

[6] Not possible due to quick pellet disintegration.

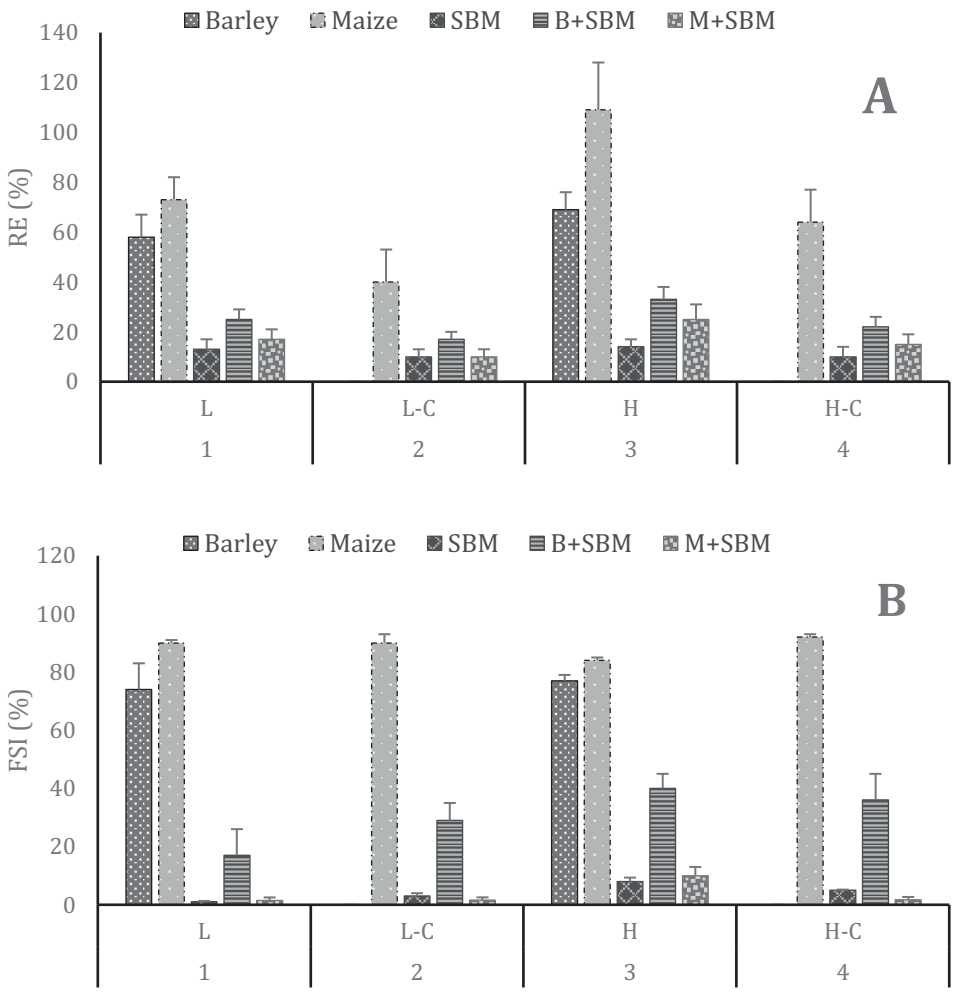


Figure 5.1 Radial expansion (RE; A) and fluid stability index (FSI; B) of individual ingredient (barley, maize and soybean meal (SBM)) and mixture (50:50, barley+soybean meal (B+SBM) and maize+soybean meal (M+SBM)) feeds produced with 2 mm screen size in hammer mill and 3 mm die size in the extruder (bars as standard deviations). At horizontal axes, upper row L is for low screw speed (210 rpm), H is for high screw speed (300 rpm), and C is for cooling applied at the last section in the extruder. Lower row, numbers 1-8 represent feed or treatment number.

Like in Paper-III, *in situ* rumen degradation of starch and protein of experimental treatments used in Paper-II was also determined, including a corresponding control treatment (expander pelleted). These measurements were made at the end of Experiment 3 using the same procedure, animals, and statistical methods as described in Paper-III. Therefore, these were not included in Paper-II, and the results are presented in the following table and figure.

Table 5.3 *In situ* rumen degradation of starch and crude protein (CP) for experimental treatments (pure barley, extruded to get three treatments with distinct densities) used in Paper II and corresponding control treatment (expander processed conventionally pelleted).

	Experimental treatments[1]					*P*-value		
Item[2]	HDcon	HDext	MDext	LDext	SEM[3]	Trt	HD × LMD	Con × Ext
Starch								
S, %	41.2^{a}	3.30^{d}	5.03^{c}	6.84^{b}	0.16	<0.01	<0.01	<0.01
Pd, %	58.3^{d}	96.6^{a}	93.8^{b}	92.2^{c}	0.18	<0.01	<0.01	<0.01
D, %	99.5^{a}	99.9^{a}	98.8^{b}	99.0^{b}	0.12	<0.01	<0.01	0.08
k_d, %	31.2^{b}	24.9^{a}	36.6^{bc}	41.1^{c}	1.98	<0.01	<0.01	0.22
ESD_5, %	91.4^{a}	83.3^{c}	87.3^{b}	88.6^{b}	0.59	<0.01	0.34	<0.01
ESD_8, %	87.6^{a}	75.9^{c}	81.7^{b}	83.5^{b}	0.82	<0.01	0.32	<0.01
Crude Protein								
S, %	24.8^{a}	7.35^{c}	9.20^{b}	9.88^{b}	0.44	<0.01	<0.01	<0.01
Pd, %	74.7^{c}	88.1^{a}	85.9^{ab}	81.5^{b}	1.23	<0.01	0.10	<0.01
D, %	99.5^{a}	95.5^{b}	95.1^{bc}	91.4^{c}	0.96	<0.01	<0.01	<0.01
k_d, %	5.7^{c}	7.68^{b}	7.46^{bc}	10.0^{a}	0.44	<0.01	<0.01	<0.01
EPD_5, %	64.5^{a}	60.5^{b}	60.4^{b}	64.1^{a}	0.66	<0.01	0.70	0.01
EPD_8, %	55.8^{a}	50.3^{b}	50.5^{b}	55.0^{a}	0.81	<0.01	0.70	<0.01

[1] HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets. HDcon was produced with the same processing settings as used in Paper-III, but barley was ground with a 6 mm screen size in hammer mill.

[2] *S*= Soluble fraction, *Pd*= Potentially degradable fraction, *D*= Potential degradability, k_d= Fractional rate of degradation of *Pd* (h^{-1}), ESD= Effective starch degradability calculated using a fractional rate of passage (k_p) of 0.05 h^{-1} (ESD_5) or 0.08 h^{-1} (ESD_8), EPD= Effective protein degradability calculated using a fractional rate of passage (k_p) of 0.05 h^{-1} (EPD_5) or 0.08 h^{-1} (EPD_8).

[3] Standard error of the mean for n=4.

[a, b, c] indicate least-square means to differ within the row.

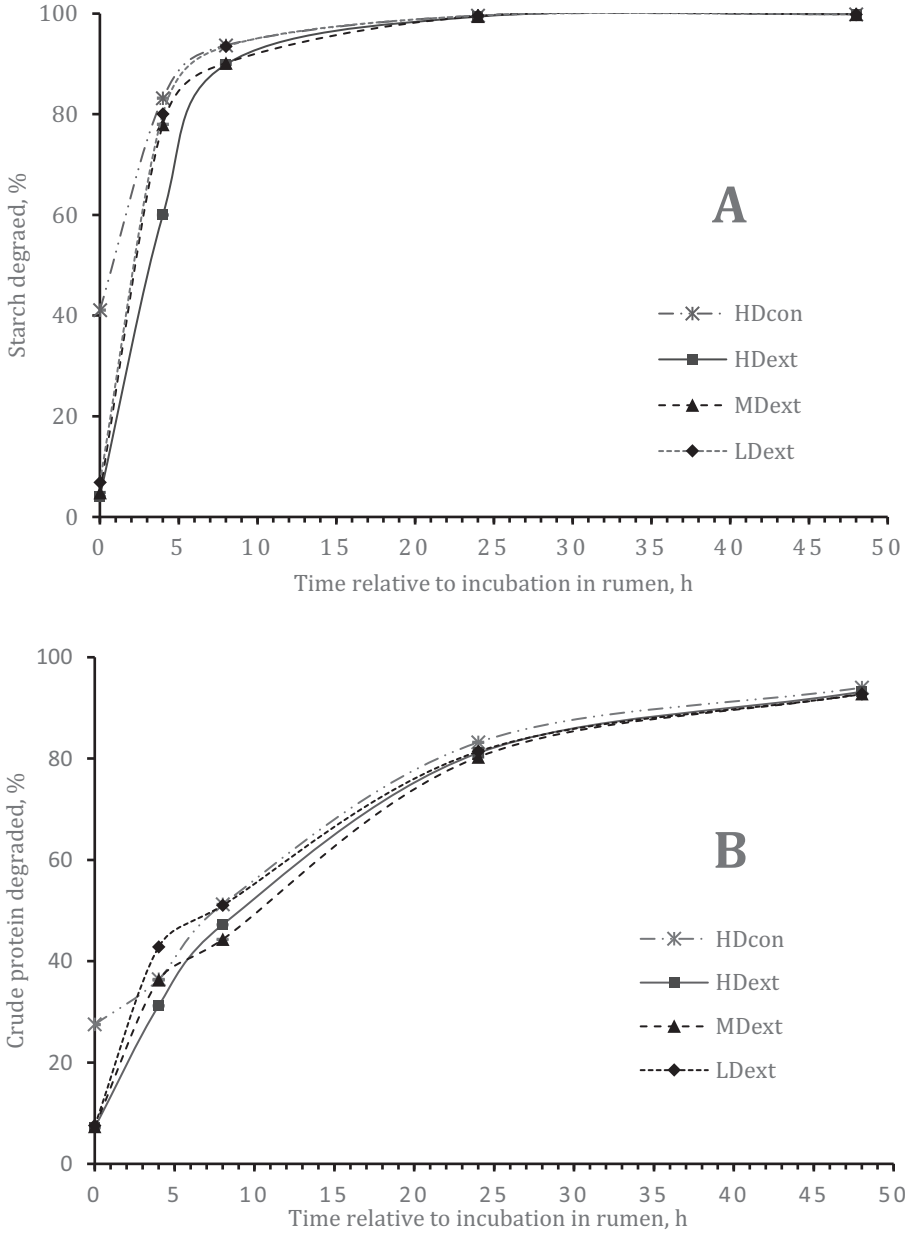


Figure 5.2 *In situ* rumen degradation profiles of starch (A) and crude protein (B) for the experimental treatments (HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets) used in Paper-II and corresponding control treatment (expander treated conventionally pelleted; HDcon). HDcon was produced with the same processing settings as used in Paper-III, but barley was ground with a 6 mm screen size in hammer mill.

6 General discussion

The overall aim of this PhD thesis was to obtain knowledge on how feed pellets with specific physical properties can be used to manipulate rumen digestion and improve utilization of starch and protein in dairy cow diets. The obtained results are discussed in detail in the three included papers. In this section main results of the papers are combined and, together with supplementary results, are discussed in a broader context.

6.1 Physical properties of feed pellets to target rumen digestion

The physical properties of feed pellets in relation to their effects on digestion behavior in the rumen are scarcely studied. Ruminal partitioning and passage of feed particles mainly depend upon their density (Sutherland, 1988). Recently, Larsen et al. (2019) studied postprandial patterns of ruminal and duodenal starch appearance in dairy cows of conventional and extruded pellets differing in the physical properties. They employed methods used in the fish feed industry to evaluate the density and water stability of pellets. However, water stability and sinking/floating properties of feed pellets are affected by agitation, temperature and ionic concentration of the medium used (Chen et al., 1999; Obaldo et al., 2002). Thus, as the rumen environment differs from seawater, the main research challenges were defining and measuring these physical properties and producing feed pellets with desired physical properties. Experiment 1 (Paper-I) was designed to evaluate how different density feed pellets behave in a rumen-like environment with respect to density and floating-/sinking properties. Moreover, as feed pellets with certain fluid stability are required to maintain density properties, the effect of rumen fluid on pellet stability was evaluated. All these effects were evaluated *in vitro* by methods used in fish feed production modified to provide a rumen-like environment as described in details in Paper-1 and shown in section 3.1.

As shown in Paper-I and supplementary results (Table 5.2 in section 5), the highest BD and SD yielding floating pellets in the rumen was 470 g/L and 0.78 g/mL, respectively. For this density, the pellets' diameter ranged from 4.2 mm to 9.14 mm, and hence density for floating was independent of the pellets' size. In fish feed, the relationship between density and sinking property of pellets is often poor if pellet size is smaller than 3 mm in diameter (Sørensen, 2012). Thus, a small pellet size (< 3 mm) may have a different density range for floating in a rumen environment. In contrast to floating pellets, the density of pellets having slow sinking, fast sinking, and very fast sinking property in the rumen was hard to identify as specific criteria do not exist. Keeping rumen volume and digesta contents in mind, an SV slower than 40 mm/sec, between 70-120 mm/sec, and faster than 120 mm/sec was arbitrarily stated for slow sinking, fast sinking, and very fast sinking pellets, respectively. SV and proportion of sinking/floating pellets varied among and

within feed materials. After the initial sinking, some slow sinking pellets were observed to float before sinking again. An explanation for this could be differences in the internal cell structure of pellets in terms of pores and voids (Piedecausa et al., 2009). However, as discussed in section 1.3.3.2, FSG of feed particles in the rumen is affected by many factors, and the density of newly ingested feed particles (or pellets) may initially increase due to hydration with rumen fluid (Hooper and Welch, 1985) and then may decrease due to adhering of gas bubbles from microbial fermentation (Wattiaux et al., 1992b).

The effect of fermentation gases on the density of pellets is complex to measure and was not determined. However, the effect of hydration on SD was determined after soaking pellets in rumen fluid for 20 min. The results revealed that SD increased more for low-density (floating) than for high-density (fast sinking) pellets. A smaller increase in SD for high-density pellets was attributed to more compaction of particles. However, despite higher compaction of particles, this increase in SD seems faster in high-density conventional pellets than in high-density extruded pellets (Paper III). This discrepancy is probably due to greater starch gelatinization (Svihus et al., 2005) and increased gluing of particles in extruded pellets compared to conventional pellets. Moreover, functional SD within rumen can also be affected by motility and digesta. Thus, based on all the above factors, a BD < 430 g/L for floating (low-density), 500-540 g/L for slow sinking (medium-density), 600-740 g/L for fast sinking (high-density), and > 740 g/L for very fast sinking (very high-density) feed pellets was suggested to obtain clear sinking and floating characteristics in the rumen (Paper-I). The corresponding SD for low-, medium-, high- and very high-density pellets was < 0.78 g/mL, 0.85-0.89 g/mL, 0.97-1.12 g/mL and > 1.12 g/mL, respectively. Similarly, to attain the required SD between 1.2 to 1.3 g/mL for rumen escape (Dufreneix et al., 2019), an SD of more than 1.05 g/mL was suggested for high-density feed pellets.

With the processing conditions tested, the required density for rumen escape can be achieved for all feed materials used, but floating pellets were obtained only for 100% cereal grains (Paper-I and Table 5.2 in section 5). However, to maintain density properties, feed pellets are required to have high fluid stability. Fluid stability of pellets for ruminant feeds is usually not determined, and, hence, the exact criterion for FSI in cattle feed pellets is unknown. Recently, Larsen and Raun (2018) found that the water stability of 24 steam pelleted commercial compound feeds for dairy cows ranged from 2 to 20% after 120 min of incubation in water at 25 °C. These values seem comparable to FSI of 20% after 90 min of incubation in rumen fluid at 39 °C for conventional feed pellets used in the present study (Paper-III). An FSI of more than 80% after incubation for 90 min in rumen fluid at 39 °C was assumed to be the optimum for feed pellets to maintain density characteristics in the rumen. Extruded pellets with 100% cereal grains met this criterion precisely (Paper-I and Paper-II). In contrast, FSI in feed pellets of protein-rich ingredients or mixtures (Paper-I and Figure 5.1B in section 5) remained below 37%. Thus, feed pellets'

desired density and fluid stability can be obtained more easily with pure cereal (starch-rich) grains than with protein-rich ingredients or mixtures under the current settings in the extruder. Nevertheless, barley and maize will require different settings in the extruder (e.g., screw speed and/or temperature in the last section of the extruder barrel) to produce feed pellets with desired density.

Starch is recognized as the major contributor of expansion and binding in the feed pellets, whereas protein and fiber are considered to reduce expansion and binding in the feed pellets (Thomas et al., 1998; Moraru and Kokini, 2003; Robin et al., 2012; Larsen and Raun, 2018). These properties of different biopolymers directly influence the physical properties of finished feed pellets. Therefore, pure cereal grains containing higher proportions of starch could produce feed pellets with high FSI and greater variability in density depending upon the operating settings in the extruder. Different processing conditions will be required to provide higher temperatures and shear forces to achieve similar effects in protein-rich and mixture feeds (Guy, 2001). Although fibers are known to reduce pellet quality (Thomas et al., 1998), the FSI of barley feeds was higher than maize feeds (Paper-I), indicating a positive effect of fibers on FSI in extruded pellets. However, FSI of barley feeds, produced with 3 mm die size in the extruder, appeared to be lower than maize feeds (75% *versus* 87%; Figure 5.1B in section 5). The exact reason behind this reduction in FSI is not known. Probably, there is some effect of die pressure (DP) on FSI as a negative correlation between FSI and DP was observed for barley feeds (Paper-I). The DP for barley feeds, specially produced with 3 mm die size, was substantially higher than for maize feeds (Table 5.1 in section 5), and consequently, high-density pellets could not be obtained with 3 mm die for barley. Thus, pure barley feeds produced with a 3 mm die size will require more die openings to reduce DP and, hence, to get feed pellets with desired FSI and density. Apart from feed material, the temperature in the last section of the extruder barrel was the most critical factor affecting the density and fluid stability of feed pellets (Paper-I). High-density feed pellets with high fluid stability can be obtained easily for pure cereals by decreasing this temperature below 100 °C through cooling of the last section of the extruder barrel.

To evaluate the effects of physical properties on dynamics of rumen digestion, feed pellets within each *in vivo* experiment were processed with, as much as possible, the same settings to reduce the potential confounding effects of processing (e.g., particle size) on digestibility. Based on Paper-I findings, experimental treatments used in Paper-II were produced successfully with barley to obtain low-, medium-, and high-density pellets with current settings in the extruder. Since the quality of 50:50 mixture feed pellets was not optimal (Paper-I), experimental treatments used in Paper-III were produced with 70% barley and 30% SBM and injecting steam at section 4 in the extruder barrel. The density and fluid stability of extruded feeds used in both *in vivo* trials were demonstrated to be

within the desired range. Thus, experimental treatments used within Paper-II and Paper-III were assumed to differ basically in the physical properties of feed pellets.

6.2 Dynamics of concentrate feed pellets in the rumen

The ruminal degradation and duodenal appearance of starch were used as indicators to evaluate the effects of physical properties (i.e., density and fluid stability) of concentrate pellets on their degradability and passage from the rumen. Pellets with high fluid stability and high density were expected to have lower rumen degradability and greater rumen outflow. This statement was partly confirmed in the conducted experiments as evidenced by a lower k_d (Paper-III and Table 5.3 in section 5) and greater duodenal starch appearance (Paper-II and Paper-III) for high-density extruded pellets than other density pellets, but the duodenal starch appearance did not differ between high-density extruded and conventional pellets, despite marked differences in FSI (Paper-III). Although ruminal degradation and passage are interrelated processes, these observations will be discussed separately in the following subsections for better elucidation.

6.2.1 Degradation of pellets

The rumen's degradation and its dynamics are determined *in situ* by nylon bag procedure and *in vivo* by rumen evacuation technique (Huhtanen and Sveinbjörnsson, 2006). Based on these measurements, high-density extruded pellets with high fluid stability showed lower k_d of starch than other pellet types (Paper III and Table 5.3 in section 5). Moreover, all extruded pellets showed a lower soluble fraction of starch than conventional pellets. Much of this measured soluble fraction is caused by small particles' loss through the bag pores (Tothi et al., 2003). Correction with initial loss of small particles was not conducted. Therefore, the highest soluble fraction for conventional pellets (HDcon in Paper-III and Table 5.3 in section 5) can be attributed to the increased loss of small particles due to their low FSI. In contrast to conventional pellets, the extent of gelatinization is great in extrusion (Svihus et al., 2005), which would glue particles together, resulting in higher FSI. This may explain the reduced k_d and soluble fraction of starch for extruded pellets. This gluing of particles was greater in high-density extruded pellets than for low and medium density extruded pellets, as indicated by a relatively high FSI for high-density extruded pellets (Paper-II and Paper-III). Thus, the lowest k_d of starch for high-density extruded pellets is observed *in vivo* and *in situ*. However, k_d of starch for low-density extruded pellets appeared to be either the same or higher than conventional pellets, despite marked differences in their FSI. As discussed in Paper III, this could be due to the longer first incubation (4h) *in situ*. Moreover, the impact of physical forces arising from digesta contents and the rumen's motility could be higher during both *in situ* and *in vivo* than during *in vitro* determination of FSI. It can be expected that the degradation of

low-density extruded pellets was probably slow during the first 2 hours, but it needs further investigation.

In the present study, intact pellets were used during the *in situ* procedure to better see the effects of the physical properties of pellets on rumen degradation kinetics. Using ground samples of either extruded (high-density or low-density) or conventionally pelleted wheat, maize, and mixtures (50:50) of them with SBM, Razzaghi et al. (2016) observed a substantially higher k_d of starch for extruded pellets than conventional pellets, which contradicts the present findings. They reported an averaged starch k_d of 89% h^{-1} for high-density and 110% h^{-1} for low-density extruded pellets compared to 46% h^{-1} for conventional pellets. Besides this, they observed much higher soluble fractions of starch, ranging from an average of 50% for extruded pellets to 79% for conventional pellets, as compared with the present study. Razzaghi et al. (2016) also did not correct for the initial loss of small particles. It is expected that grinding of pellets likely disrupted particles' binding and exposed more starch granules to microbial breakdown, yielding higher soluble fraction and k_d of starch. This demonstrates that using ground or intact feed pellets during *in situ* determination would yield substantial differences in rumen degradation kinetics, particularly for extruded pellets.

Apart from the direct estimation, the differences in the k_d of starch are reflected by the patterns or ratios in which VFA (acetic, propionic, and butyric acids) are produced in the rumen (France and Dijkstra, 2005). Because of differences in density and FSI, it was expected that pellets would provide different ruminal pH and VFA patterns after feeding. In this regard, low-density extruded pellets with the lowest likelihood of passing out the rumen were thought to have more fermentation in the dorsal and medial rumen than high-density extruded pellets. Moreover, conventional pellets with high density and low FSI were assumed to degrade in the ventral rumen resulting in local acidic conditions compared with extruded pellets. Hence, extruded pellets may beneficiate the rumen environment more than conventional pellets. However, except for high propionate concentration in the dorsal rumen for low-density pellets (Paper-III), no clear patterns for fermentation indicated that low-density pellets were fermenting more towards dorsal rumen than high-density pellets (Paper-II and Paper-III) or conventional pellets were fermenting more in the ventral rumen than extruded pellets (Paper-III). Rumen fermentation varies more when the concentrate is fed before forage (Voigt et al., 1978). Therefore, feeding of concentrate before forage could have provided a better opportunity to observe the effects of feed pellets on rumen fermentation patterns. Using the same feed pellets as Razzaghi et al. (2016), Larsen et al. (2019) studied the effects of density and water stability on intra-ruminal mixing and postprandial duodenal starch appearance in dairy cows. They also did not observe any clear patterns for rumen fermentation variables among the pellet types. Effects of mixing contractions of the rumen and, as Larsen et al. (2019) indicated, animal-to-animal variation in speed of moving ingested concentrate

pellets within the ruminal cavity could be the possible reasons for unclear fermentation patterns among pellets types. It is suggested that multiple subsamples from the sampled ruminal plane may reduce animal-to-animal variation (Larsen et al., 2019).

Contrary to expectation, extruded pellets, despite having a high FSI, showed a lower rumen pH than conventional pellets (Paper-III). This pH drop was exceptionally high with low-density pellets, which could be due to their high k_d of starch as observed *in situ* and *in vivo*. This trend in pH drop was not observed in Paper-II. However, the acetate:propionate ratio was lower for low-density pellets than high-density pellets (LD *versus* HD in Paper-II), which corresponds with k_d of starch observed *in situ* for these pellets (Table 5.3 in section 5). Hence, the assumption that extruded pellets with low density and high stability will ferment slowly in the dorsal rumen providing elevated rumen pH is not proved under the current experimental conditions. A higher concentration of butyrate in the ruminal fluid was observed for conventional pellets than for extruded pellets (Paper-III). This agrees with Prestløkken and Harstad (2001), who observed increased butyrate concentration in rumen fluid for expander pelleted compared with ordinary pelleted barley-based diet. Under certain conditions, concentrate diets may encourage the development of a large protozoal population, accompanied by an increase in butyrate rather than propionate (France and Dijkstra, 2005).

6.2.2 Outflow of pellets

The passage of starch from the rumen is generally assumed to follow first-order kinetics with an exponential decline. However, patterns of postprandial duodenal starch flow (Paper-II and Paper-III) indicate that starch passage may deviate from first-order passage kinetics, as also observed by Tothi et al. (2003). The duodenal starch flow appeared to be delayed for feed pellets based on their density and fluid stability characteristics. The starch flow from low- and medium-density pellets was delayed, probably, due to their lower SD and high FSI. For high-density extruded pellets, it was delayed probably to attain optimal SD (1.2 to 1.3 g/mL) for rumen escape, as their initial SD was 1.05 g/mL. SD determined in rumen fluid demonstrated that they could attain the required density after 20 min in the rumen. However, the SD of feed pellets may continue to be less than the optimal for some time under gas evolution from microbial fermentation (Sutherland, 1988). Thus, high-density pellets might have taken more time *in vivo* to overcome this effect. Another possibility could be that these pellets have left the rumen early but started accumulating in the abomasum. The passage from the abomasum decreases with density (Faichney, 1986) because particles in the abomasum must be pushed up against their tendency to settle. When a certain amount of these pellets was accumulated in the abomasum, they were pushed upward (push-effect) through the pylorus towards the duodenum. The delayed starch flow was more pronounced when bigger pellets (produced with 6 mm die size) were used (Paper-II) where duodenal starch

flow increased at a relatively lower rate than observed in Paper-III and by Larsen et al. (2019). This caused more starch flow towards evening than after morning allocation of pellets (Paper-II). The extruded pellets used by Larsen et al. (2019) and in Paper-III were produced using a 2.4 mm, and 3 mm die size, respectively, and therefore it is likely that size of pellets did not differ much between studies. Thus, although the relationship between particle size and passage is indecisive (as discussed in section 1.3.3.3), it appears that the size of feed pellets can have a considerable impact on pellet flow dynamics from the rumen, especially for highly stable extruded pellets.

Fluid stability is essential for the physical integrity of feed pellets (Welker et al., 2018) and hence to maintain density properties in them. Therefore, despite having high density, conventional pellets were expected to have decreased passage from the rumen due to low FSI compared with high-density extruded pellets. However, in contrast to density, the fluid stability appeared to have none or limited effect on rumen outflow of feed pellets as high-density conventional pellets with low FSI (20%) (HDcon in Paper-III) showed a similar duodenal starch appearance as high-density extruded pellets with high FSI (84%). Larsen et al. (2019) observed a greater duodenal starch appearance for conventional pellets with high water stability (ranging from 47% to 98%) than with low water stability (3%). This led them to suggest that feed pellets with high density and liquid stability can have higher rumen escape. However, this was not confirmed in the present study, although starch flow patterns were different between high-density conventional and high-density extruded pellets (Paper-III). There might be some other factors affecting the passage of high-density conventional pellets, like apparently quick rate of hydration (as discussed in section 6.1), which may increase the chances of settling these pellets in the reticulum by quickly attaining optimum density. The rate and capacity of water adsorption in feed meal is mainly governed by chemical composition and particle size, both increasing with decreasing particle size (Hemmingsen et al., 2008). Although a screen size of 3-4 mm in a hammer mill is usually recommended for ruminant compound feeds, the conventional pellets used in the present study (Paper-III) and by Larsen et al. (2019) were produced using a 2 mm screen size. Thus, producing conventional feed pellets from a feed meal with smaller particles may have higher rumen outflow than feed pellets produced from a meal with coarser particles. Nevertheless, the passage of particles from the rumen is not only affected by intrinsic feed characteristics but also by factors like DMI, amount of particles in the rumen, ruminal contractions, and physical and physiological status of the cow (Kennedy, 2005).

The eating behavior for high-density extruded pellets varied among cows, which can influence these pellets' outflow from the rumen. When postprandial duodenal starch flow was determined for two cows eating all the experimental concentrates within 15 min after allocation, high-density extruded pellets showed significantly higher flow than other pellet types ($P_{Trt} = 0.01$). Moreover, differences between high-density conventional pellets

and low-density extruded pellets were reduced (Figure 6.1). Hence, high-density extruded pellets might have higher rumen escape compared to conventional pellets than observed. This needs further investigation on cows eating similarly.

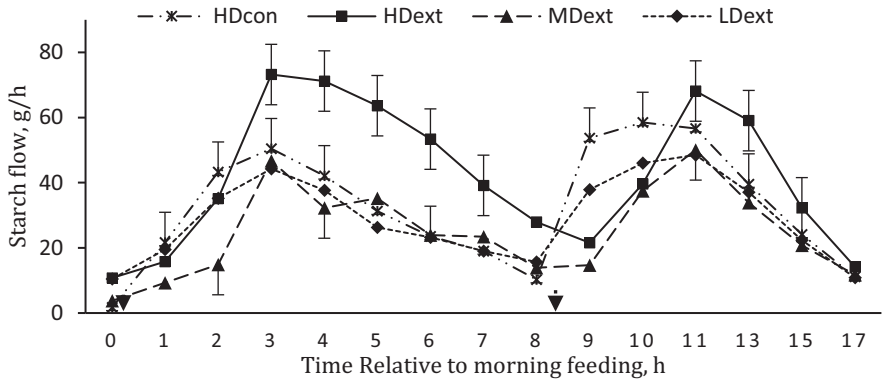


Figure 6.1 Postprandial duodenal flow of starch for experimental concentrates (HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets) for two cows consuming all the concentrates completely within 15 min after allocation. The solid arrow indicates morning feeding, and the dashed arrow indicates afternoon feeding of concentrates.

In contrast to the present study, Larsen et al. (2019) could not observe any clear pattern for postprandial starch flow among pellet types despite marked differences in bulk densities of extruded pellets. However, BD of low-density extruded pellets in their study averaged 477 ± 36 g/L, which is the upper limit for floating pellets in the present study (Paper-I and Table 5.2 in section 5). In addition, as discussed in Paper-I, the correlation between BD and SD for high-density extruded pellets is poor. SD of feed pellets was not determined by Larsen et al. (2019), and probably the SD of their high-density extruded pellets was below the optimum SD for rumen escape. Thus a higher density for low-density extruded pellets and a likely suboptimal SD for high-density extruded pellets were probably the reasons for no differences in duodenal starch flow between low-density and high-density extruded pellets by Larsen et al. (2019). Hence, suggested BD of maximum 430 g/L (or SD < 0.78 g/mL) for floating pellets and an SD of more than 1.05 g/mL for high-density pellets, to have increased rumen passage, confirmed to be optimal. Moreover, the present study revealed that, although BD is more easily determined, it could be challenging to produce feed pellets for increased rumen escape based only on BD, and it is crucial to determine SD in addition.

6.3 Effects on digestibility of starch and protein

The assimilation of starch and protein in the ruminant is the net result of the ruminal and post-ruminal digestion, where rumen digestion is characterized by the concurrent rate of degradation and rate of passage. Thus, high-density extruded pellets with lower k_d

and higher rumen passage showed lower RSD than other density pellets (Paper-II and Paper-III). Barley starch is considered readily digestible in the rumen with an average digestibility of around 87% (Nocek and Tamminga, 1991; Huntington, 1997), but with variation depending upon differences between barley varieties, amount of starch intake, DMI, degree of processing, and feeding level. By conducting a meta-analysis of starch digestion in lactating cows, Moharrery et al. (2014) found an average RSD of 89% (ranging 85-92%) for processed barley in their database where barley starch had made up more than 95% of total starch intake. Although extruded pellets were produced with similar processing conditions as possible, low-density pellets had higher processing temperatures than high-density pellets, which may lead to increased starch gelatinization and, consequently, increased k_d and extent of digestion (Svihus et al., 2005). However, as discussed in Paper-III, steam processing does not have any notable impact on RSD for readily digestible feedstuffs like barley and wheat (Mills et al., 1999a). Thus, a higher RSD for low-density pellets was primarily due to their reduced probability for rumen escape. A similar RSD for medium-density extruded pellets supports this, although the processing temperature was lower in medium-density than in low-density extruded pellets. Moreover, conventional pellets appeared to have a similar k_d of starch as low-density extruded pellets, but their RSD was reduced likely due to a high passage, as discussed above. Despite this, the effect of different processing temperatures, during the production of concentrate pellets with differing physical properties, on RSD needs to be evaluated in future studies.

Since the passage of conventional pellets was rapid, their RSD did not differ from high-density extruded pellets (Paper-III). Postprandial duodenal starch flow indicates that almost all the ingested starch was either digested or escaped from the rumen up to 17 h after morning feeding. Therefore, RES can be calculated from the postprandial duodenal starch flow, and it was 20, 20, 12, and 13% for conventional, high-density extruded, medium-density extruded, and low-density extruded pellets respectively (Paper-III). Interestingly, this calculated RES was higher than RES obtained from a daily duodenal starch flow. Possible reasons can be a high number of samples (15 *versus* 8), increased amount of the concentrate fed per feeding (5 kg *versus* 3.3 kg), and use of 100% experimental concentrate for postprandial duodenal starch flow. Similarly, when RES was calculated for two cows with normal intake, it was 15, 20, 12, and 14% for conventional, high-density extruded, medium-density extruded, and low-density extruded pellets, respectively. Thus, it can be speculated that RSD with high-density extruded pellets could have been reduced more if all cows had a similar eating pattern and that these may provide at least 25% more metabolizable starch than conventional pelleting. Indeed, this is based on only two cows and should be investigated further.

Overall, RSD was lower in Paper-II than Paper-III (82% *versus* 89% for extruded pellets) and the reported average RSD for barley, even though starch outflow rates were

slow in Paper-II. DMI does not seem to vary greatly between the two experiments, and dietary starch concentration was quite constant at 150 g/kg DM. Apart from several other factors that could affect RSD (Mills et al., 1999a), a reduced RSD in Paper-II was mainly due to the relatively high FSI of feed pellets and was further reduced by the large size of disintegrating particles from feed pellets. The feed meals were ground with a 6 mm and 2 mm screen size in hammer mill in Paper-II and Paper-III, respectively. It can be expected that there might be significant differences in particle size distribution in treatments used in two experiments, as can be envisaged by differences in mean particle size in Paper-I. However, conventional pelleting has been observed to reduce particle size, where this effect was higher on coarse particles than on small particles (Khan and Prestløkken, 2015). Likewise, extrusion pelleting can also reduce particle size, thereby decreasing differences in particle size distribution between two grindings. The particle size distribution of concentrate pellets was not determined. However, visually examining the detached particles during the fluid stability test revealed that extruded pellets produced with 6 mm screen size in hammer mill have a higher proportion of coarse particles than with 2 mm screen size. It has been observed that RSD decreases with an increase in particle size when comparing milling by grinding and rolling (Larsen et al., 2009).

The total tract digestibility of starch was more than 99% in both *in vivo* experiments, indicating the post-rumen starch digestion was not hampered. Since the small intestine is the only site where glucose absorption occurs, starch escaping the rumen should preferably be digested therein. However, as discussed in section1.2.2, the efficiency of SISD could be limited by insufficient time for digestion and/or inadequate access to the enzymes (Owens et al., 1986). As more than 98% of ingested starch was digested up to distal ileum without any significant differences among the treatments (Paper-II and Paper-III), it is evident that SISD was not limited to a greater extent under current conditions. Many studies have reported a negative correlation between intestinal starch digestion (ISD) and the amount of starch escaping the rumen (Nocek and Tamminga, 1991; Offner and Sauvant, 2004; Moharrery et al., 2014). In the current study, when such correlation was computed collectively for both experiments (Paper-II and Paper-III), it somewhat agrees with previous findings but non-significant (Figure 6.2). Nevertheless, within experiments, this correlation appeared to be positive. For the same amount of starch entering the intestine, ISD was lower in Paper-II than in Paper-III. These differences are probably due to differences in particle size of RES, as discussed above for RSD. This indicates that the physical accessibility of starch by pancreatic amylase is the main limiting factor determining SISD. This agrees with Larsen et al. (2009), who indicated that the same factors limit the action of bacterial and pancreatic amylase. When concentrate pellets were produced with smaller particle size (Paper-III), ISD (g/kg RES) was increased with the increasing amount of RES, making a positive correlation between ISD and RES (Figure 6.2). Hence, amylase production/secretion and brush border α-glucosidases

activity may not be the major limiting factors for SISD in dairy cows. However, it is important to note that dietary starch concentrations and consequently RES concentrations in duodenal digesta were low in the current study. The negative correlation, typically observed between ISD and RES, is more pronounced when RES is higher than 100 g/kg DM (Offner and Sauvant, 2004). The dietary starch concentration usually ranges between 200 to 300 g/kg DM for lactating cows, where starch intake can be up to 11 kg/d (Mills et al., 1999a). Since the increase in starch intake reduces RSD (Moharrery et al., 2014), the amount of RES can likely increase with the increase in starch intake, and hence ISD may decrease. Nevertheless, Figure 6.2 shows that high-density pellets with low RSD can be digested more efficiently in the small intestine when they are produced with smaller particle size.

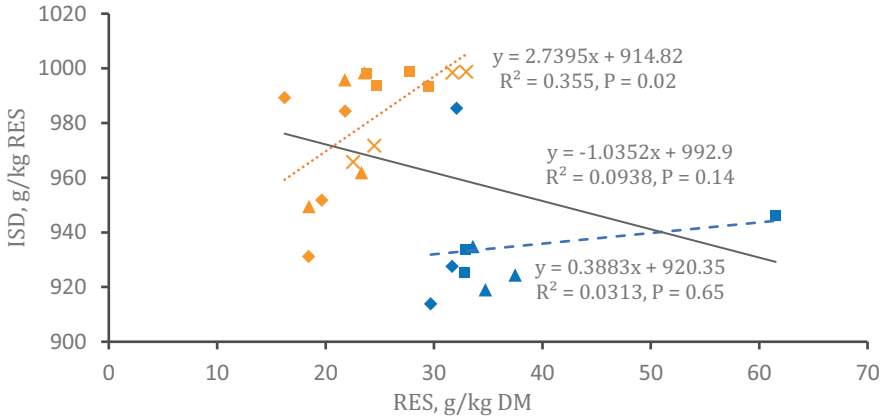


Figure 6.2 Correlation between rumen escape starch (RES, g/kg DM) and intestinal starch digestibility (ISD, g/kg RES). Blue markers are for Paper-II, where feed material was ground by 6 mm screen size, and orange markers are for Paper-III, where feed material was ground by 2 mm screen size in hammer mill. (×), (■), (▲), and (◆) represents HDcon, HDext, MDext, and LDext, respectively. The solid line represents the overall correlation between the two experiments.

In contrast to starch, the crude protein did not provide any clear digestion pattern with respect to the physical properties of feed pellets based on *in situ* and *in vivo* results. Based on RSD kinetics, a lower ruminal degradation and higher rumen outflow of dietary crude protein could be expected for high-density extruded pellets, especially in Paper-III, where 30% SBM was included in the compound meal. By considering the apparent CP digestibility in the small intestine in Paper-II, it can be expected that a higher proportion of dietary protein might have escaped rumen digestion for high-density extruded pellets. However, this statement is not supported by Paper-III, where the duodenal flow of CP appeared to increase with the decrease in feed pellets' density. Direct estimation of the proportion of dietary CP degraded in rumen and intestine was not conducted. Usually, a high correlation is seen between *in situ* degradation and *in vivo* measurement of duodenal CP flow (Volden, 1999; Prestløkken and Harstad, 2001). Comparing *in situ* EPD (Table 5.3

in section 5 and Paper-III) with corresponding *in vivo* duodenal flow of CP (Paper-II and Paper-III), it could be expected that a higher proportion of dietary CP might have escaped rumen for high-density extruded pellets in Paper-II and for low-density extruded pellets in Paper-III. It seems that, contrary to the assumption, protein in concentrate pellets might not follow the patterns of starch digestion even though both entities were present in the same pellet. However, the contribution of concentrate protein in the duodenal flow was hard to decide since no further indications from protein metabolites in rumen fluid and N utilization were seen (Paper-III). Moreover, as discussed in Paper-III, an increased duodenal CP flow, particularly for low-density pellets, could also be due to increased microbial protein synthesis due to a better synchronization of the rumen release of nutrients. Despite all these uncertainties, it is interesting to see a greater supply of metabolizable protein with extruded pellets than conventional pellets, which needs proper evaluation in future studies.

6.4 Effects on fiber digestibility

Since roughages are the fundamental component of the dairy cow's diet, the maximal digestibility of fibers is vital to improving milk yield, the efficiency of feed utilization, and animal health. The ruminal and total tract digestibility of fibers has been observed to decrease with an increase in the allocation of rapidly degraded starch (McCarthy et al., 1989; Overton et al., 1995; Sadri et al., 2009). This decreased fiber digestion with an increased supply of starch is mainly attributed to lower rumen pH. Cellulolytic bacteria are more sensitive to low pH than amylolytic bacteria, and the pH below 6 is recognized to impair the growth of cellulolytic bacteria (Russell and Wilson, 1996). Ruminal pH decreases rapidly with the increase in the rate of starch digestion. Thus, it was expected that extruded pellets would improve the utilization of forages by benefiting the rumen environment either by partly shifting the site of starch digestion (high-density pellets) or slowly fermenting in the dorsal rumen (low-density pellets) compared with conventional pellets. In both *in vivo* experiments, apparent ruminal and total tract digestion of NDF did not differ among the pellet types (Paper-II and Paper-III). However, contrary to expectation, the rumen digestion of NDF was lower with extruded pellets than with conventional pellets (Paper-III). It seems that ruminal NDF digestibility decreased linearly with the increase in RSD as the density of extruded pellets decreased. However, this trend was not seen in Paper-II. A low NDF digestibility with extruded pellets could be due to lower rumen pH than conventional pellets (Paper-III), but corresponding increased duodenal CP flow with extruded pellets does not support that low pH has any major negative impact on microbial growth. Nevertheless, low rumen pH may not be the only causative factor for decreased fiber digestibility (Huhtanen et al., 2006). There were no correlations between ruminal pH and NDF digestibility within both experiments (Figure 6.3B). Similarly, correlations between RSD and NDF were non-significant within

experiments (Figure 6.3A). Despite lower pH in the dorsal and medial rumen, ruminal NDF digestibility was high (> 70%) in Paper-II, and DMI was not affected among pellet types in Paper-III, indicating that extruded pellets did not compromise rumen function. There could be some other unfavorable conditions causing decreased rumen NDF digestion with extruded pellets in Paper-III.

One possible reason could be the effect on rumen fill, which is usually associated with depression in feed intake. DMI was not affected in the present study, but rumen volume was numerically higher for extruded pellets than conventional pellets (Paper-III). When cows were challenged with rumen fill in the form of dietary NDF (by increasing 25 to 35% in diet) and inert bulk, rumination activity, frequency of reticular contractions, and k_p of NDF from the rumen increased, but DMI was decreased only at 35% NDF with inert bulk (Dado and Allen, 1995). In addition to lag time in the passage, extruded pellets, especially low-density pellets, might have increased the rumen raft's buoyancy, which in turn could have increased ruminal contractions by exerting pressure on the rumen walls and thus increased digesta outflow. This could explain increased duodenal DM flow for low-density pellets in postprandial samples, particularly after morning feeding (Paper-II and Paper-III). However, differences in ruminal NDF digestion were compensated by hindgut fermentation. This explains that if not beneficial, extruded pellets do not significantly negatively impact fiber utilization. Nonetheless, a decreased ruminal NDF digestibility, particularly with low-density pellets either by impeding cellulolytic bacteria' growth or increasing ruminal contractions, is unclear and needs further investigation.

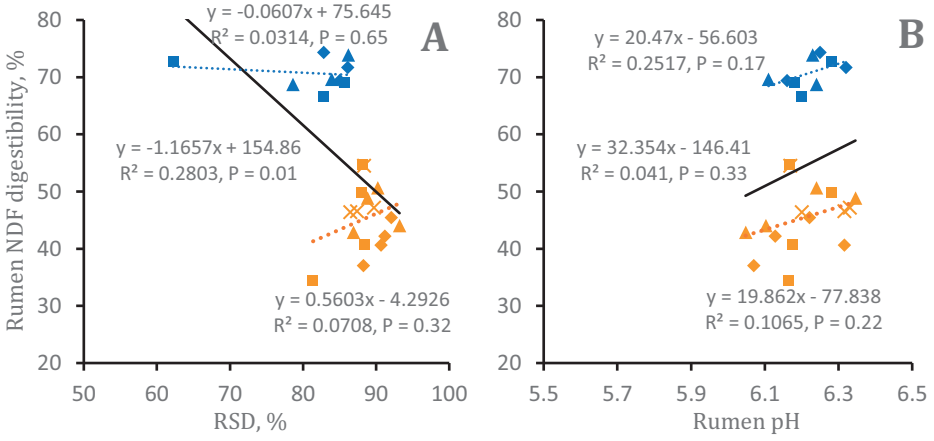


Figure 6.3 Correlations of rumen NDF digestibility (%) with rumen starch digestion (RSD, %; A) and rumen pH (B). Rumen pH is the average of all observations (postprandial and diurnal) pooled into cow and period. Blue markers are for Paper-II, whereas orange markers are for Paper-III. (×), (■), (▲), and (◆) represents HDcon, HDext, MDext, and LDext, respectively. The solid line represents the overall correlation between the two experiments.

Regardless of the pellet types, NDF digestibility was lower in Paper-III than Paper-II. This decreased fiber digestibility apparently seems to be the effect of higher RSD.

However, as indicated above, NDF digestibility can be affected by several other intrinsic and extrinsic factors (Huhtanen et al., 2006). Of these, plant species and the indigestible fraction of NDF (iNDF), which determines the intrinsic rate and extent of fiber digestion, are the major factors affecting the utilization of forages in ruminants. The iNDF fraction not only decreases the extent of NDF digestion but may also increase the passage of NDF, although digestible NDF can selectively be retained in the rumen (Allen and Mertens, 1988; Huhtanen et al., 1995). Besides, the passage of particulate matter increases with the increase in the dietary concentration of NDF (Bosch et al., 1992; Dado and Allen, 1995). The increased passage rate will reduce rumen NDF digestibility. Silage used in Paper-II comprised perennial ryegrass and white/red clover. In contrast, silage in Paper-III was a mixture of mainly timothy and meadow fescue with some red clover. Moreover, the characteristics of silages differ between both experiments. The iNDF, chewing time, and rumen fill were higher, being 216 g/kg NDF, 73 min/kg DM, and 0.54 /kg DM, respectively in Paper-III compared with 134 g/kg NDF, 44 min/kg DM, and 0.39 /kg DM in Paper-II (data provided by Eurofins analysis). In addition to high RSD, these differences may explain lower fiber digestion in Paper-III than observed in Paper-II.

6.5 Challenges, limitations, and implications

A few challenges were encountered when investigating the effects of physical properties of feed pellets on utilization of starch and protein in dairy cows, which may be relevant for future studies.

1) Production of 50:50 mixture feed pellets with desired physical properties was impossible to achieve under current extruder operating settings. The possible reasons can be moderately cooking screw configuration and, as indicated in Paper-I, decrease in melt temperature and shear due to starch-protein interactions (Allen et al., 2007). To keep confounding effects of processing minimal between the two *in vivo* experiments, this problem was partly solved by increasing the proportion of starch in the mixture (i.e., using 70% barley instead of 50%) and partly by slightly modifying the extruder settings (i.e., applying steam in the extruder for low-density extruded pellets) (Paper-III). The temperature and shear can be increased by applying steam directly into the extruder or/and increasing screw speed. However, these approaches may help little as, for example, increasing screw speed decreases residence time and hence cooking (Lin et al., 1997). An alternative can be changing screw configuration with more mixing and kneading elements.

2) With the application of cooling at the last section in the extruder to obtain high-density pellets, the temperature also decreased in the previous sections (Paper-I). This can reduce cooking of feed material and thus fluid stability of feed pellets, especially in compounds feeds containing a higher proportion of protein ingredients. This effect was probably due to the low feed rate (100-150 kg/h), as the extruder's maximum capacity was 800 kg/h.

Hence, using a higher feed rate may solve this problem. Alternatively, using a "two-step" extrusion process, where one extruder on the top for cooking and another for shaping the pellets, may further improve pellet quality, particularly FSI in high-density extruded pellets.

3) The intake of extruded pellets, especially high-density pellets, was challenging. The addition of molasses usually improves the palatability of feed pellets (Spörndly and Åsberg, 2006), but it did not help here. The low palatability of extruded pellets appeared mainly due to their high hardness, as cows were observed to have difficulty chewing these pellets. The hardness of conventional and high-density extruded pellets, when measured with a flat knob, was almost similar but with knife knob hardness of high-density extruded pellets was markedly higher than conventional pellets (Paper-III). It demonstrates that feed pellets' chewability can be better assessed by determining the hardness with a knife knob instead of a flat knob. Extruded pellet's intake was considerably improved by producing on a 3 mm die size instead of 6 mm. However, the palatability of extruded pellets in cattle needs proper investigation.

4) There is a balance between chewing and not chewing of feed pellets. Too much chewing before swallowing can also be a limitation regarding density and disintegration. This limitation may be reduced by using smaller size feed pellets. Given this limitation and the problem with intake, a die size of 3-4 mm seems suitable for extruded pellets intended for use in cattle.

Dairy cows are commonly fed total mixed rations (TMR) or partially mixed rations (PMR) offered from a feed bunk. Alternatively, and common in Norway, forage and the concentrate are fed separately. Feeding concentrate pellets with specific physical properties as mixed ration will not be an adequate way of feeding as feed pellets may lose their structure due to hydration and mechanical breakdown of mixing before eaten by a cow. Hence, these special pellets should be fed separately either in the milking unit or in the concentrate stations. In Norway, this will not be a big problem since most dairy farms feed concentrate and forage separately or offer a proportion of concentrate in a milking robot. However, as discussed above, the palatability of high-density extruded pellets can be a challenge. This can have severe implications on cow traffic when extruded pellets are fed in milking robots. Apart from producing pellets with a suitable smaller size, intake problems can also be reduced by feeding these extruded pellets mixed with commercial compound pellets.

7 Concluding remarks and future perspectives

The main conclusion to be drawn from the present study is that the dynamics of rumen digestion of compound feeds can be manipulated by producing feed pellets with specific physical properties. In this regard, the density of feed pellets appeared to be the main property determining the passage and, hence, the rumen's digestion. High-density feed pellets had higher rumen outflow than other density pellets. However, the concept that high-density extruded pellets with high fluid stability will have greater rumen escape than conventional pellets was not supported by this thesis's work. However, k_d of starch was low with high-density extruded pellets indicating that fluid stability can impact the rate of degradation of feed pellets. Moreover, no evidence was found that extruded pellets will beneficiate the rumen environment for fiber digestion more than conventional pellets. In contrast to starch, the crude protein did not provide any clear pattern of rumen digestion with respect to the physical properties of feed pellets even though both entities were present in the same pellet. Furthermore, the study revealed that concentrate feed pellets with high fluid stability and different sinking characteristics in the rumen could be achieved by extruder processing.

Improving the utilization of starch and protein in compound feeds through extruded feed pellets with specific physical properties is a novel concept. Although no major breakthrough has been found in the present study, there were some indications that high-density extruded pellets can provide more metabolizable energy than conventional feed pellets, requiring further investigations. Moreover, it seems feasible to use low-density (floating) extruded pellets to prompt a better ruminal N and energy synchrony for improving MCP yield and N utilization, but the direct measurement of MCP yield is needed. Hence, the full potential of the approach used in this thesis has yet to be uncovered.

Feed is a complex substance consisting of several polymer types. Interactions between/among operating conditions and feed ingredients during processing are the major impediments to tailor-make concentrated feed pellets with required physical functional properties. Although attempts were made in the present study to reduce potential confounding factors during feed processing, it is likely that factors such as levels of starch gelatinization among feed pellet types may differ due to different processing conditions, which may confound the results. Thus, future studies must consider the potentially confounding roles of feed processing during the production of feed pellets intended to have different physical properties. Apart from feed production challenges, the complexity of the rumen digestive process creates obstacles to achieving the desired effects of feed pellets with respect to the rumen and intestinal digestion of starch and protein. More research is needed to explore further the relation between pellet properties and pellet behavior in the rumen.

Extruder cooking is an expensive production method due to high investment costs and relatively low production capacity than conventional steam pelleting or expander pelleting. Therefore, lactation performance studies with milking cows need to be conducted to evaluate the economic feasibility of using extruder feed processing for dairy cows. However, it would be worth investigating if the high-density feed pellets (with a greater likelihood of rumen escape) can be produced using conventional pelleting methods. In this regard, the effects of different particle sizes of feed mash on the physical properties of pellets in relation to their behavior in the rumen (as suggested in the present study) should be explored in future studies. In addition, conventional pellets produced by using pellet binders (like lignin-based or others) to improve the fluid stability of feed pellets and their effects on rumen escape should be evaluated. Moreover, investigating alternative feed production methods to obtain high-density pellets with high fluid stability would be interesting. These could be the semi extruder/expander methods available through machines like the "Crown expander" from Kahl (A. Kahl GmbH, Reinbek, Germany) or the Universal pellet cooker (UPC) from Wenger (Wenger Inc., Sabetha, KS, USA).

8 References

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Paper-I

Targeting nutrient utilization in ruminant diets through extruder processing: Production and measurement of physical properties of feed pellets

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Abstract

The study was designed to investigate if extruder processing could be used to produce feed pellets with physical properties targeted to affect the probability of rumen escape. The physical properties were evaluated using laboratory methods. The feed materials used were barley, maize, soybean meal (SBM), barley + SBM (B+SBM; 50:50), and maize + SBM (M+SBM; 50:50). The processing conditions used were two settings in a hammer mill (feed materials ground with either 2 mm or 6 mm screen size) and four extrusion settings (screw rotation speed either 210 rpm or 300 rpm, and application of cooling or not in the last section of the extruder barrel) using the twin-screw extruder. The physical properties studied were radial expansion (RE), bulk density (BD), sinking velocity (SV), specific density (SD), and fluid stability index (FSI). The study revealed that maximum BD for floating pellets was 469 g/L (SD 0.76 g/mL) and minimum BD for fast sinking pellets was 570 g/L (SD 0.96 g/mL), whereas pellets with BD 502 g/L (SD 0.85 g/mL) were slow sinking. The SD of pellets increased after immersion in rumen fluid from 0.006 g/mL to 0.31 g/mL, where this increase was higher for low-density pellets than for high-density pellets. Feeds from barley and maize gave highly stable pellets with an average FSI of 89 ± 7%, whereas SBM feeds provided pellets with low FSI (average 8 ± 3%). Mixture feeds were also less stable, giving an FSI of 22 ± 11%. The type of feed material and cooling at the last section in the extruder barrel were the most critical processing parameters affecting feed pellets' density and fluid stability, followed by screw speed and particle size of feed materials. Overall, maize gave the highest RE and consequently lowest densities with more floating feeds. Maximum RE was achieved for the feed ground at 2 mm screen size and extruded at 300 rpm without cooling in the last section. Cooling in the last section of the extruder barrel decreased RE in all feeds, giving high-density pellets with fast SV. The highest density pellets were

obtained for the feed ground at 2 mm screen size and extruded at 210 rpm. From this research, it can be concluded that density and fluid stability of feed pellets from pure cereal grains can be easily targeted by manipulation of screw speed and temperature in the last section of the extruder barrel, whereas feeds containing a high proportion of protein ingredients will require other processing settings and needs further investigation.

Abbreviations: BD, bulk density; DP, die pressure; FSI, fluid stability index; MPS, mean particle size; RE, radial expansion; SBM, soybean meal; SD, specific density; SD_{rf}, Specific density of pellets in rumen fluid; SME, specific mechanical energy; SV, sinking velocity; T3, temperature in section 3 of extruder barrel; T5, temperature in section 5 of extruder barrel; WSI, water stability index

Keywords: In vitro; Extrusion; Density; Fluid stability; Ruminants

1. Introduction

High-producing dairy cows need a good balance between nutrients digested in the rumen and the small intestine to meet their nutritional requirements and ensure efficient feed utilization. Rumen digestion and rumen escape of starch and protein are of particular importance in this respect. In theory, rumen digestion of starch and protein is determined by the rate of rumen degradation and rate of rumen passage. The rate of rumen digestion has been intensively studied, and knowledge on ingredient differences and effects of processing on starch and protein has been used to design feeds for ruminants for decades. In contrast, the influence of ingredient and processing on the rumen passage rate of starch and protein is scarcely studied.

A prerequisite for rumen escape is that feed particles can leave the rumen before the rumen microbes digest them. In order to escape the rumen, feed pellets, like all other feed particles, need to be present in the reticulum from where the rumen passage takes place. The presence of feed particles in the reticulum is mainly dependent on their density (Lechner-Doll et al., 1991; Offer and Dixon, 2000). Using plastic particles, desBordes and Welch (1984), Ehle and Stern (1986), and Murphy et al. (1989) showed that high density (1.17 and 1.42 g/mL) particles have a higher probability for rumen escape than low density (< 1 g/mL) particles. Later, these ranges have been confirmed by Seyama et al. (2017) and Dufreneix et al. (2019). Hence, feeding pellets with optimal high density could increase rumen escape. In addition to increasing the probability of rumen escape of nutrients like starch, high-yielding cows may also need nutrients digested over time in the rumen to ensure optimal utilization without digestive disturbances like acidosis. A slowly degradable floating pellet (low density) may have less probability of rumen escape but may provide an optimal balance between nutrient demand and nutrient release. Thus, manipulating passage properties of

feed pellets by targeting their densities could be an approach for increasing feed utilization in dairy cows.

Steam pelleting, where feed particles are agglomerated into a dense pellet, is the most commonly used processing method for compound feeds to dairy cows. Expander pelleting is an alternative to steam pelleting, where feed is processed under additional temperature and pressure. Physical properties of pelleted feeds for ruminants are generally evaluated concerning pellet hardness and durability. Recently, it has been demonstrated that such conventionally pelleted feeds typically have high density and low water stability (Larsen and Raun, 2018). Water stability is closely linked to pellet disintegration (Welker et al., 2018). Therefore, conventional pellets may disintegrate rapidly in the rumen, thereby losing their density properties.

A third method for feed processing is extrusion cooking, a thermo-mechanical process where the pre-conditioned mash is cooked and forced through a die at the extruder outlet (Miladinovic and Zimonja, 2010). During extrusion cooking, molecular transformations like starch gelatinization and protein denaturation convert feed material into a viscoelastic dough or melt where droplets of water are entrapped under the presence of high temperature, pressure, and shear. As the melt exits the outlet die, steam flashes off, forming a porous and expanded pellet. This phenomenon imparts characteristic physical functional properties like density, durability, hardness (Sørensen, 2012; Khater et al., 2014), and high water stability (Welker et al., 2018) in extruded pellets.

The physical properties of pellets are strongly influenced by choice of feed material and processing parameters, e.g., particle size, screw configuration, screw speed, heating or cooling of the extruder barrel (Camire, 1998; Chevanan et al., 2008; Sørensen et al., 2010; Ayadi et al., 2013). Therefore, effects of various feed formulations and extrusion operating parameters have been broadly studied to achieve pellets with targeted physical properties like high water stability and various sinking

velocities in seawater (Rolfe et al., 2000; Chevanan et al., 2007; Sørensen et al., 2010; Draganovic et al., 2011; Kraugerud et al., 2011; Fallahi et al., 2013), and extrusion has become the primary technique for the production of aquaculture feeds. To what extent extrusion can be used to target physical properties to affect rumen digestion and passage kinetics of starch and protein in pelleted feeds for ruminants is to our knowledge not studied, except for Larsen et al. (2019).

The main objective of this experiment was to investigate if extruder processing could be used to produce feed pellets with high fluid stability and varying in density for sinking/floating behavior in rumen fluid for manipulating probability of rumen escape and synchronizing nutrient demand and release. Also, as most tests to describe the physical properties of feed pellets have been adapted to needs in the fish feeding systems, and since the rumen environment differs from the sea, an additional objective of the research was to define how to measure the physical properties of feed pellets for ruminants.

2. Materials and Methods

2.1. Feed ingredients and processing of extruded feeds

All feeds were processed at the Center for Feed Technology (FôrTek) at the Norwegian University of Life Sciences, Ås, Norway. Barley, maize, and soybean meal (SBM; solvent extracted obtained from Denofa AS, Fredrikstad, Norway) were used either individually or in a mixture to form a total of 40 extruded test feeds. Feed production was split into two trials using the same processing conditions.

The experiments were conducted in complete factorial designs, i.e., 3x2x2x2 and 2x2x2x2 for the first trial to give 24 feeds and the second trial to give 16 feeds, respectively. The factors studied were feed material, screen size in a hammer mill (2 mm or 6 mm), extruder screw speeds (210 rpm

or 300 rpm), and cooling at the last section in the extruder barrel (Yes or No). In the first trial, the individual ingredients barley, maize, and SBM were processed. Due to the adverse quality of pellets obtained in pre-trial testing of 100% SBM, 10% maize was included in SBM. In the second trial, mixtures of barley+SBM (B+SBM; 50:50) and maize+SBM (M+SBM; 50:50) were processed. The chemical composition and mean particle size (MPS) of feed materials used are shown in Table 1. MPS was determined as a geometric mean diameter according to ASABE (2013) by using the dry sieving technique. The process settings used in the first and second trials are shown in Table 2 and Table 3, respectively.

Barley, maize, and SBM were ground separately in a hammer mill (HM 21.115, Münch-Wuppertal, Germany) using 2 mm and 6 mm screen sizes. After milling and batching, all individual ingredients and mixtures were mixed in a twin shaft paddle mixer (Forberg AS, Larvik, Norway) for 120 seconds. Mash was pre-conditioned similarly for all feeds in a double conditioner (BCTC 10, Bühler, Uzwil, Switzerland) with a constant feeder rate of 100 kg/h. About 18 ± 1 kg/h moisture was added in the conditioner as liquid (58.7 ± 5% of added H_2O) and steam (41.3 ± 5% of added H_2O) to get a total moisture content of 29 ± 1% and a temperature between 85 and 90 °C. The pre-conditioned mash was extruded in a co-rotating twin-screw extruder (Bühler BCTG 62/20 D; 5 barrel's sections) by using 6 mm die size (revolver die; six number of dies). Four extruder operating parameters were used. They were low (210 rpm) and high (300 rpm) screw speeds combined with either cooling (to control exit temperature between 80-90 °C) or without cooling of the last section (section 5) in the extruder barrel. The screw length was 1260 mm, and the screw configuration was 100R100-100R100-80R80-80R80-P120-60L20 (90° twist-off)-80R80-80R80-80R80-80R80-80R60-60R60-60R60-60R60-60R60-60R60-60R60-20R60-40R60, where the first number represents the pitch length, the second number the length of the screw element, and R and L

indicate forward and backward conveying direction. The P120 is a polygonal kneading element having forward conveying properties.

The extruded pellets were dried in a fluid bed continuous dryer (FôrTek, NMBU) for 7 to 10 min at approximately 100 °C to achieve a final moisture content of a maximum of 12%. Samples were taken after the production steady-state was achieved and cooled in batch coolers (FôrTek, NMBU). Extruder data was recorded, and extruder barrel temperature at section 3 (T3) and section 5 (T5), die pressure (DP), torque, and specific mechanical energy (SME) are reported for individual ingredients in Table 2 and mixtures in Table 3.

2.2. Analysis of physical properties

Samples collected were subjected to analyses of physical properties in the form of radial expansion (RE), bulk density (BD), sinking velocity (SV), specific density (SD), and fluid stability index (FSI). These analyses were with some modifications based on procedures used in the fish farming industry and are presented in detail below.

2.2.1. Radial Expansion (RE)

RE was determined by measuring the diameter of randomly selected pellets at three different points. The average pellet diameter was used to calculate expansion (%) by the following formula: $RE = \frac{Dp-De}{De} \times 100$ Where (Dp) is average pellet diameter, and De is the die size in the extruder. The reported value is an average of 30 measurements for each feed sample.

2.2.2. Bulk density

BD was determined as described by Sørensen (2012). In this method, pellets are poured into a one-liter tared steel cylinder without agitating. Excess pellets are removed by a scraper, gently pulling

over the edge of the cylinder. The cylinder filled with pellets was then weighed, and BD (g/L) was measured three times for each feed sample.

2.2.3. Sinking velocity (SV)

SV of pellets was determined in rumen fluid, collected from two rumen-cannulated cows fed a standardized diet at the maintenance level. The fluid was mixed and strained through a 200 µm mesh cloth (SEFAR NITEX, Sefar AG, Heiden, Switzerland). A 250 mL transparent glass cylinder (310 mm long and 35 mm inner diameter) was used, having two fixed points marked 220 mm apart with 30 mm of fluid column above and below these points. The cylinder was filled with filtered rumen fluid and placed in an incubation cabinet at a temperature of 42 °C to ensure a rumen fluid temperature between 38-39 °C. The temperature of rumen fluid in the glass cylinder was frequently monitored by a thermometer. A lamp was placed behind the glass cylinder to illuminate the rumen fluid to ease pellet movement observation. Randomly selected pellets were then dropped one by one from a height of about 30 mm above the fluid surface, and SV was determined as mm/sec by measuring the time elapsed to travel the distance of 220 mm, using a manually operated stopwatch. For each feed sample, randomly selected 30 pellets were tested.

A supply of rumen fluid was stored at a constant temperature, and rumen fluid was renewed in the glass cylinder after 10 pellets measurements. The density of rumen fluid was also measured at 39 °C before testing for each feed sample, and it remained constant at 0.988 ± 0.001 g/mL.

2.2.4. Specific density (SD)

SD, also known as geometric envelop density of pellets, was calculated using the following formula: $SD = \frac{Wp}{Vp}$ Where Wp is the weight of pellets and Vp is the volume of the same pellets.

Pellet weight was determined using a laboratory scale (AG204 DeltaRange®, Mettler-Toledo GmbH, Greifensee, Switzerland).

To accurately determine the volume of irregular-shaped extruded pellets, the volumetric displacement method was used. The method was modified from the method initially developed by Hwang and Yakawa (1980) by using a tapped density analyzer (Thomas, 2004). Since glass beads' penetration into extrudates can affect the volume determination (Joardder et al., 2015), glass beads with 0.5 mm diameter were used as displacement medium in a 10 mL or 25 mL graduated glass cylinder depending upon the size of the pellets. Pellets were selected randomly, but very few pellets with visibly open pores were discarded.

In short, 5-10 selected pellets were weighed (Wp) together. Then, the volume of glass beads (Vi) was measured in a graduated glass cylinder without pellets by tapping 100 times with AUTOTAP (AUTOTAP, Quantachrome Instruments, 1900 Corporate Drive, Boynton Beach, Florida, USA). After that, glass beads were taken out of the glass cylinder, and some were poured back into making a thin layer in the bottom of the cylinder. A pellet was placed on top of the layer, and enough glass beads to cover the pellet were poured into the cylinder. This process was continued until all the weighed pellets were covered in glass beads. Finally, the remaining glass beads were poured on top, and the cylinder was tapped 100 times again to get the final volume (Vf) of pellets and glass beads. The volume of pellets (Vp) was calculated as $Vp = Vf - Vi$ after which SD of pellets in g/mL was calculated using the previously described equation. Each value reported was an average of five measurements per feed sample.

SD was also determined after immersion in rumen fluid to investigate the change in the density of pellets. 5-10 selected pellets were soaked in rumen fluid at 39 °C for 20 min. After soaking, pellets were placed on tissue paper to absorb excess water on the pellets' surface. Thereafter, SD was

156 determined as described above and denoted as "SD_{rf}" i.e., SD of pellets in rumen fluid. The
157 reported value is the average of three measurements.

158 *2.2.5. Fluid stability index (FSI)*

159 The fluid stability was determined by modifying the water stability index (WSI) method of
160 Baeverfjord et al. (2006), developed for testing fish feeds. The main modifications were rotational
161 agitation, higher temperature, and ruminal fluid as a medium instead of tap water. Thus, the name
162 fluid stability index (FSI) was given instead of WSI.

163 FSI was determined using ball-shaped stainless steel baskets (Anping Amma Filter Equipment
164 Co., Ltd., Hengshui City, China) having an inner diameter of an average 58 ± 2 mm, filtered rumen
165 fluid, and the Daisy^{II} Incubator (ANKOM Technology, Fairport, NY, USA). The original size of
166 the basket mesh was 0.7 mm. To enhance the removal of disintegrating particles, holes of 2 mm
167 were made manually with an awl. About 5 g of pellets were weighed in a basket, and the basket
168 was closed tightly using a clip attached to it. The rumen fluid was collected and processed as
169 described for SV. Two liters of rumen fluid were poured into a daisy incubator glass jar, fitted with
170 two small bulges to ensure baskets' twirling during rotation. Three baskets carrying three different
171 feed samples were placed in each glass jar and wholly immersed in rumen fluid. Then, glass jars
172 were placed in the Daisy^{II} Incubator at 39 °C and rotated at a speed of five rotations per min. Each
173 basket was twirled 10 times per min. After 90 min, baskets were removed from the glass jars. After
174 removing excess fluid, the baskets were cleaned outside gently with tissue paper, weighed, and
175 placed in an oven at 103 °C for 18 h. The pellet stability was calculated as dry matter retained after
176 incubation in rumen fluid divided by dry matter before incubation. The results are reported as an
177 average value of three replicates within the feed.

178 **2.3. Statistical Analysis**

179 Pearson product-moment correlation procedure in SAS (SAS, 2013) was used to check inter-
180 relationships between independent and dependent variables and among dependent variables. The
181 dependent variables were extruder process variables (T3, T5, DP, torque, and SME) and pellets'
182 physical properties (RE, BD, SV, SD, and FSI). Independent variables were processing conditions
183 (screen size in hammer mill, screw speed, and cooling at last section in the extruder). The results
184 were presented as correlation coefficients (r) and considered significant at $P < 0.05$ and as a
185 tendency at $0.05 > P \leq 0.10$.

186 The feeds were produced in continuous runs without replicates. However, within feeds, physical
187 quality was measured several times and considered as repeated measurements. Thus, the MIXED
188 procedure and repeated measurement statement of SAS (2013) was used to evaluate treatment
189 effects on RE, BD, SV, SD, and FSI of pellets according to the following model:

190 $$Y_{ijklm} = \mu+\alpha_i+\beta_j+\gamma_k+\delta_l+\alpha_i\times\beta_j+\alpha_i\times\gamma_k+\alpha_i\times\delta_l+\beta_j\times\gamma_k+\beta_j\times\delta_l+\gamma_k\times\delta_l+\alpha_i\times\beta_j\times\gamma_k+\alpha_i\times\beta_j\times\delta_l+\alpha_i\times\gamma_k\times\delta_l+\eta_m+e_{ijklm}$$

191 here Y_{ijklm} is the dependent variable; μ is the overall mean of the dependent variable; α_i is the effect
192 feed material; β_j is the effect of screen size in hammer mill; γ_k is the effect of screw speed; δ_l is the
193 effect of cooling at the last section; η_m is the random effect of feed; e_{ijklm} is the random errors
194 associated with observation *ijklm*. Two-way interactions were used for all main effects, whereas
195 three-way interactions were used only, including feed material. The variance-covariance (VC) was
196 used as a covariance structure for repeated measurements. The significance level of each factor
197 was determined by Kenward-Roger denominator degrees of freedom approximation for the type
198 III test of fixed effects resulting from the model where restricted maximum likelihood (REML)
199 was used as an estimation method. The least-square (LS) means ± standard error of the mean

(SEM) of main effects and two-way interactions involving feed material are presented. Multiple comparisons were tested using PDIFF statement and considered significantly different at $P < 0.05$ and as a tendency at $0.05 < P \leq 0.10$.

3. Results

The feed materials provide different MPS under similar screen sizes in hammer milling. As expected, larger MPS were obtained with a 6 mm screen size. Barley and B+SBM gave the largest MPS, 823 µm and 692 µm, respectively. With decreasing screen size, MPS was reduced (by 43%) to a greater extent for barley than for maize and SBM. MPS of maize, SBM, and M+SBM were below 568 µm.

3.1. Process variables during extrusion.

The extruder temperatures varied greatly among the individual ingredients (Table 2) but not between the mixtures (Table 3). The highest temperature was observed for maize sample no. 7 without cooling at the last section, having 130 and 133ºC for T3 and T5, respectively. T3 was correlated with T5 for all feeds (r = 0.893, P<0.001, n=40). Cooling applied at last section significantly decreased T3 (r = -0.694, P<0.001, n=40) and T5 (r = -0.933, P<0.001, n=40).

THE lowest DP was observed for maize and M+SBM feeds (Table 2 & Table 3). DP decreased with an increase in screen size in the hammer mill, giving a negative correlation between two variables for barley, B+SBM, and M+SBM (r = -0.726, P<0.001, n=24). The DP was negatively correlated with T5 for all feeds (r = -0.353, P=0.025, n=40). DP increased with increase in fiber content (r = 0.799, P<.001, n=40).

During the extrusion of individual ingredient feeds, the torque was lower in barley feeds than maize and SBM feeds (Table 2). Torque was lower in mixture feeds than individual ingredient

feeds, and it was higher in B+SBM feeds than M+SBM feeds (Table 3). With the increase in screw speed, torque decreased; however, the correlation was significant only for maize, SBM, and M+SBM (r = -0.694, P<0.001, n=24). Torque tended to be negatively correlated with screen size in hammer mill (r = -0.466, P=0.068, n=16) and cooling at last section (r = -0.501, P=0.053, n=16) for barley and B+SBM feeds. There was a positive correlation between torque and DP for all feeds (r = 0.773, P<0.001, n=32) except maize feeds (r = 0.099, P=0.815, n=8).

SME increased with increase in screw speed (r = 0.552, P<0.001, n=40). When cooling was not applied at last section in extruder barrel, SME was positively corelated with T3 and T5 (r = 0.808, P<0.001, n=20). SME was correlated with DP, negatively for maize feeds (r = -0.736, P=0.036, n=8), but positively for all other feeds (r = 0.601, P=0.001, n=32). Overall, SME was positively correlated with torque for maize, SBM and M+SBM (r = 0.682, P<0.001, n=24).

3.2. Physical properties of pellets

3.2.1. Radial Expansion (RE)

RE varied among feed materials and processing conditions used in both trials (Figure 1; Table 6 and Table 9). For individual ingredient feeds, RE was 80% higher for cereal grains than SBM, where it was 49% higher for maize than for barley (Table 7). For mixture feeds, B+SBM expanded 22% more than M+SBM (Table 10). RE was about 22% higher for feeds produced with 2 mm than with 6 mm screen size in hammer mill in both trials. Due to interaction between feed material and screen size in hammer mill, the above effect of smaller screen size on increasing RE was higher for barley in individual ingredient feeds (Table 8) and B+SBM in case of mixture feeds (Table 11). However, the correlation between screen size in hammer mill and RE was significant only for barley (r = -0.802, P=0.017, n=8). In both trials, RE increased on average by 27 ± 3% by increasing

screw speed from 210 rpm to 300 rpm and decreased by 52 ± 4% with cooling at the last section in the extruder barrel. These effects were higher for maize followed by barley, whereas SBM remained unaffected concerning individual ingredient feeds (Table 8). For mixture feeds, the effect of increasing screw speed on increasing RE was higher for B+SBM than for M+SBM (Table 11). RE increased with increase in SME (r = 0.607, P=0.001, n=32) and T5 (r = 0.660, P<0.001, n=32) for all feed materials except for SBM. For maize and M+SBM, there was a negative correlation between DP and RE (r = -0.902, P<0.001, n=16), whereas for barley and B+SBM, this correlation tended to be positive (r = 0.439, P=0.089, n=16). A significant (P = 0.017) interaction between screen size in hammer mill and screw speed (Table 6) indicates that RE will increase with a smaller screen size in hammer mill and higher screw speed in the extruder. RE was correlated positively with starch (r = 0.679, P<0.001, n=40) and negatively with protein (r = -0.618, P<0.001, n=40) and fiber (r = -0.634, P<0.001, n=40) contents.

3.2.2 Bulk density (BD)

The BD varied from 429 g/L to 627 g/L for barley, 284 g/L to 835 g/L for maize, 626 g/L to 698 g/L for SBM, 568 g/L to 659 g/L for B+SBM and 651 to 744 g/L for M+SBM (Table 4). BD increased with decrease in RE for all feed materials (r = -0.803, P<0.001, n=32) except for SBM. The BD was significantly affected by feed material for both individual ingredient feeds (Table 6), and mixture feeds (Table 9). It was higher for SBM than barley and maize (Table 7) and M+SBM compared with B+SBM (Table 10). BD decreased by 3% for individual ingredient feeds with the increase in screen size in the hammer mill (Table 7). BD decreased by increasing the screw speed and increased when cooling was applied at the last section for both individual ingredient and mixture feeds (Table 7 and Table 10). In contrast to RE, screen size in hammer mill and screw speed affected BD similarly for barley and maize, but BD in SBM remained unaffected (Table 8).

By cooling the last section in the extruder barrel, BD increased to a greater extent for maize, followed by barley and SBM relative to the feeds produced without cooling. For all feed materials except SBM, BD decreased with increase in SME (r = -0.490, P=0.004, n=32) and T5 (r = -0.765, P<0.001, n=32).

3.2.3. Sinking velocity (SV)

The SV of feeds ranged from floating to slow sinking (20 – 41 mm/sec) to fast sinking (74 – 112 mm/sec) for barley (Table 4). Maize gave either floating or fast sinking feeds (100 – 180 mm/sec). For SBM (104 to 114 mm/sec), B+SBM (70 to 114 mm/sec) and M+SBM (80 to 156 mm/sec), mostly fast sinking feeds were observed. SV was strongly correlated with BD (r = 0.923, P<0.001, n=40) and thus RE (r = -0.775, P<0.001, n=40). The effect of screw speed with either cooling or without cooling at the last section in the extruder barrel varied largely for barley, giving feeds with various SV. Without cooling at the last section, maize gave only floating, whereas, with cooling, it yielded only fast sinking feeds irrespective of screw speed. SBM and mixture feeds did not show such large variations in SV and were mostly fast sinking (Table 8 and Table 11).

3.2.4 Specific density (SD)

The SD ranged from 0.76 g/mL to 1.10 g/mL for barley, 0.43 g/mL to 1.23 g/mL for maize, 0.94 g/mL to 1.04 g/mL for SBM, 0.89 g/mL to 1.08 g/mL for B+SBM and 1.00 g/mL to 1.18 g/mL for M+SBM (Table 5). The overall correlation between BD and SD was positive and high (r = 0.959, P<0.001, n=40). Like BD, SD increased with a decrease in RE for all feed materials (r = -0.889, P<0.001, n=32) except for SBM. The mean SD was significantly affected by feed material in the case of individual ingredient feeds (Table 6), but, for mixture feeds, it tended to be affected by feed material (Table 9). SD was higher for SBM than barley and maize; however, in contrast to

BD, SD was lower in maize than barley (Table 7). SD decreased by 8% with increasing screw speed from 210 rpm to 300 rpm and increased by 27% when cooling was applied at the last section for individual ingredient feeds (Table7), but not for mixture feeds (Table 10). In contrast to BD, increasing the screw speed decreased SD in maize but not in barley and SBM (Table 8). By cooling the last section, SD increased to a greater extent in maize than in barley feeds. SD was not affected by cooling in SBM feeds. For all feed materials except SBM, SD decreased with increase in SME (r = -0.514, P=0.003, n=32) and T5 (r = -0.810, P<0.001, n=32).

SD increased for all feeds after soaking in rumen fluid at 39 °C for 20 min (Table 5). This increase in SD ranged from 0.006 g/mL to 0.31 g/mL, where it was the highest for feeds with low SD (Figure 2). For the same SD, this increase in SD was the highest for barley and the lowest for M+SBM feeds.

3.3.5 Fluid stability index (FSI)

Barley and maize showed higher FSI than SBM and mixture feeds (Figure 3). When comparing barley and maize, FSI decreased with increase in RE (r = -0.805, P<0.001, n=16), but for B+SBM and M+SBM the correlation between RE and FSI tended to be positive (r = 0.432, P=0.094, n=16). For individual ingredients, FSI was significantly affected by feed material and cooling at the last section in the extruder (Table 6). The highest FSI was observed for barley and the lowest for SBM (Table 7). Increasing screw speed decreased FSI by 7%, whereas application of cooling at the last section increased FSI by 12% for maize (Table 8). FSI of barley and SBM remained unaffected by screw speed and cooling at the last section in the extruder. For mixture feeds, the inclusion of SBM strongly reduced the FSI for both barley and maize (Figure 3; Table 10). A significant correlation (P <0.05) between FSI and DP was observed for all feed materials except SBM. This correlation was positive for maize (r = 0.745, n=8) and negative for barley (r = -0.838, n=8), B+SBM (r = -

312 0.717, n=8) and M+SBM (r = -0.865, n=8). FSI increased with increased amount of starch (r =
313 0.679, P<0.001, n=40), but decreased with increased amount of protein (r = -0.625, P<0.001,
314 n=40).

315 **4. Discussion**

316 *4.1 Behavior of extruded pellets in rumen fluid.*

317 In the rumen, feed particles are separated according to their sedimentation/floatation properties
318 (Sutherland, 1988). Low density (< 1 g/mL) particles float in the dorsal compartment of the rumen.
319 In contrast, particles having a density higher than 1 g/mL sediment towards the ventral
320 compartment with sinking velocities depending upon their densities. Thus, since SV of feed pellets
321 mainly depends on pellets' density (Chevanan et al., 2007; Sørensen, 2012), the effect of density
322 on SV in rumen fluid can be an excellent criterion to rank feeds with respect to floating or sinking
323 behavior in rumen environment.

324 The SV of pellets for ruminant feeds has not been determined before. However, it is frequently
325 determined in fish feeds where SV of pellets is commonly measured in tap water and a column
326 height of 500 to 1500 mm. Typically, SV of slow-sinking salmon feeds range from 55 to 155
327 mm/sec (Chen et al., 1999; Piedecausa et al., 2009). Since temperature and salinity affect water
328 density and thereby the sinking velocity of pellets (Chen et al., 1999), we used rumen fluid at cow
329 body temperature (39 °C) instead of water at 25 ºC to determine SV. Moreover, a column height
330 of 220 mm was used. We defined feeds with SV below 40 mm/sec as slow sinking, feeds with SV
331 between 70 to 120 mm/sec as fast sinking, and feeds with SV above 120 mm/sec as very fast
332 sinking in the rumen. However, the rumen environment is not only fluid and contains fibers that
333 can trap feed pellets. Thus, a sinking floating pattern seen *in vitro* may not happen in the rumen.

In the present study, SV was strongly correlated with BD and SD. Thus, as BD is more easily determined than SD, BD is probably the physical property to aim for as it can be easily measured during feed processing. Based on our observations, clear sinking and floating characteristics of feed pellets in the rumen can be obtained at a BD < 430 g/L (low-density) for floating, 500-540 g/L (medium-density) for slow sinking, 600-740 g/L (high-density) for fast sinking and > 740 g/L (very high-density) for very fast sinking pellets. The corresponding SD for low-, medium-, high- and very high-density pellets is < 0.78 g/mL, 0.85-0.89 g/mL, 0.97-1.12 g/mL and > 1.12 g/mL, respectively. Despite strong overall correlations, SV varied for the same BD or SD among feed materials and the feeds within the same feed material, particularly for high-density pellets. As SV can also be affected by pellets' porosity (Piedecausa et al., 2009), this could be attributed to differences in pellets' internal cell structure in terms of pores and voids. The pellets with the same density can have different cellular structures (Kristiawan et al., 2016). This also explains why some feeds showed greater variability in SV and proportion of sinking/floating pellets than others in rumen fluid.

Only three feeds produced pellets with SD over the minimum suggested density (1.17 g/mL) for increased rumen passage based on experiments with plastic particles (desBordes and Welch, 1984; Seyama et al., 2017). However, in contrast to plastic particles, feed pellets are not biologically inert, and their density may change in the rumen. The density of newly ingested feed particles initially increases due to hydration with saliva and rumen fluid (Hooper and Welch, 1985). Subsequently, it may decrease due to the adhesion of gas bubbles from microbial fermentation (Wattiaux et al., 1992). Therefore, Dufreneix et al. (2019) suggested that a density between 1.2 to 1.3 g/mL would be optimum for the increased passage of particles from the reticulorumen. Since the density of newly ingested feed particles increases due to hydration in the rumen, SD_{rf} was

357 determined after immersion in rumen fluid for 20 min. It appeared that the SD of pellets increases
358 on average 0.16 ± 0.15 g/mL. However, this change in SD was minor for high SD pellets (Figure
359 2), probably due to more particles' compaction. In addition, hydration can also increase the volume
360 of extruded pellets (Piedecausa et al., 2009), which can counterbalance the effect of hydration on
361 increasing density. However, this effect seems less contributing, as evident by a greater increase
362 in SD for pellets with low SD. A higher increase in SD for low SD pellets could also be due to an
363 increase in flexibility of these pellets by soaking and thereby compression by the added glass beads
364 giving lower volume. Furthermore, Chen et al. (1999) and Vassallo et al. (2006) observed an
365 increase in pellets' weight but no change in pellets' dimensions after immersion in water for up to
366 15 min. In the current study, 20 min of soaking was used based on a maximum increase in SD for
367 low SD (< 0.78 g/mL; floating) pellets. Despite a higher increase in SD for these pellets, their SD_{rf}
368 remained below 1 g/mL (or below the density of rumen fluid, i.e., 0.988 g/mL), and thus, the
369 density suggested for floating pellets is optimal. In contrast to low-density pellets, high-density
370 pellets will require more time to get fully hydrated and to have maximum increase in SD. Keeping
371 in view a longer hydration time for high-density pellets and a possible increase in volume of
372 pellets, it can be expected that feed pellets with SD of more than 1.05 g/mL could attain the
373 required SD for increased rumen passage after some time in the rumen. However, this lower limit
374 of SD and time required in rumen to attain optimal density for escape may differ between feed
375 materials as, in addition to internal cell structure of pellets, water uptake is also affected by the
376 chemical composition of the feed material (Ramanzin et al., 1994). Thus, a higher increase in SD
377 of barley feeds indicate that high density pellets from barley will require less time to attain required
378 density for rumen escape compared with other feed materials used in the present study.

Larsen et al. (2019) studied the postprandial duodenal starch appearance of extruded pellets, having either low-density (LD) or high-density (HD), and conventional pellets of wheat, maize, and mixtures (50:50) of them and SBM. They could not observe any significant difference in postprandial starch flow among pellet types, despite marked differences in the densities of extruded pellets as BD ranged from 428 to 516 g/L for LD and 633 to 701 g/L for HD pellet types. However, they did not determine the SD of the pellets. In the present study, when pellets with BD from 633 to 701 g/L were compared with their respective SD, the SD ranged from 0.97 to 1.10 g/mL, and the correlation between BD and SD was not significant (r=0.254, P=0.362, n=15), although the overall correlation was highly significant. This indicates that high BD pellets used by Larsen et al. (2019) might have SD below optimum for increased rumen passage, which may partly explain why they could not observe any increased postprandial starch flow for HD pellets compared with LD pellets. Thus, although BD is more easily obtained, it could be challenging to produce feed pellets for increased rumen escape based only on their BD.

To maintain the effect of feed pellet density on rumen passage, certain stability in rumen fluid is required. Assuming that pellets with FSI more than 80% after incubation for 90 min could be considered highly stable, all feeds containing 100% cereal grains, except one for maize, met that criteria. In contrast, SBM and all mixtures feeds had low fluid stability. Larsen and Raun (2018) found that the WSI of 24 steam pelleted commercial compound feeds for dairy cows ranged from approximately 2 to 20% after 120 min incubation, using the method of Baeverfjord et al. (2006). In contrast, Larsen et al. (2019) observed WSI ranging from 47 to 98% for steam-pelleted feeds, except for 100% maize, where WSI was only 4%. For extruded feed containing 100% cereal grains, they observed an average WSI of 83 ± 6%. However, their extruded feed mixtures had an average WSI of 72 ± 9%, which is relatively high compared to the FSI of feed mixtures in the present

study. These differences could be due to differences in processing conditions and methods to determine pellets stability between the two studies. WSI of feed pellets is affected by agitation, temperature and ionic concentration of the medium used (Obaldo et al., 2002); therefore, we determined FSI using more vigorous agitation and rumen fluid at 39 °C instead of water at 25 °C to imitate the effects of reticulorumen contractions and digesta contents on pellet stability. However, fluid stability of pellets for ruminant feeds is usually not determined and, hence, exact criteria for FSI in cattle feed pellets is not known.

At the extruder settings used and combining density, sinking velocity, and fluid stability, only barley and maize were able to give feeds meeting the requirements of sinking for increasing the probability of rumen escape and floating for potentially improving the synchronization of nutrients demand and release in our experiment. However, slow sinking feeds were only obtained for barley, whereas only maize gave feeds with very high density. In this respect, a very high-density pellet may attain a density above 1.4 g/mL, causing feed pellets to stay longer in the ventral sac, thereby reducing rumen escape (desBordes and Welch, 1984; Dufreneix et al., 2019). However, to achieve this, pellets will need to remain intact for a pretty long time, which probably can rarely occur in a rumen environment.

4.2 Factors affecting the density and fluid stability of extruded feed pellets.

Among the parameters studied, feed material and cooling at the last section in the extruder barrel were the most critical factors affecting the density and fluid stability of feed pellets, whereas extruder screw speed and screen size in hammer milling were identified as factors of less importance. Feed material used contained different proportions of starch, protein, and fiber, which exhibit different functional properties during extrusion (Guy, 2001). These biopolymers impart specific rheological properties to the viscoelastic extrusion melt, which directly influence flow

dynamics and process responses during extrusion and the quality of the finished product (Forte and Young, 2016). The extrusion melt's viscosity is an essential factor in determining the extrudate expansion (Kristiawan et al., 2016) and, thus, the textural properties of extruded pellets.

When cooling was not applied at the last section in the extruder barrel, the increased RE, and consequently decreased density, in maize could be attributed to its higher starch content. The density of extrudates is strongly correlated to expansion and changes in cell structures, pores, and voids developed during extrusion processing (Patil et al., 2005). Starch is recognized as the major player in the extrudate expansion (Moraru and Kokini, 2003). It undergoes several structural changes during extrusion, including gelatinization, melting, and fragmentation (Lai and Kokini, 1991). The starch gelatinization during extrusion cooking favors the expansion (Gomez and Aguilera, 1984) by forming a matrix trapping water vapors, which forms air bubbles due to a drop in external pressure upon die exit. The increased starch conversion leads to lower melt viscosity, which promotes mobility and increases the rate of bubble growth (Moraru and Kokini, 2003). Thus, starch gelatinization was presumably higher in maize promoting RE. High temperatures and SMEs observed for maize further support this as both are directly related to increased starch cooking during extrusion (Diosady et al., 1985). In contrast to maize, barley contains a higher fraction of fibers. In addition to diluting starch content, high contents of fibers reduce RE by decreasing starch conversion through reduced water binding, affecting the air bubble formation and growth (Robin et al., 2012) in barley. Probably, due to this balancing effect of fibers on RE, barley was able to give pellets with a range of densities from floating to slow sinking to fast sinking.

Although temperatures and SMEs were similar between SBM and maize, SBM could not produce pellets with large density variations. Proteins also undergo similar changes as starch and yield a

plasticized fluid mass during extruder processing (Forte and Young, 2016). However, proteins require processing conditions that include higher moisture levels, temperatures, and shear forces (Guy, 2001), which were probably not achieved for SBM feeds during the current processing settings. Despite higher starch concentration, mixture feeds behave more towards SBM, giving mostly high-density pellets. Lower temperature and SME for mixture feeds than individual ingredients may explain this trend, presumably resulting in reduced starch gelatinization and reduced RE. These lower temperatures and SMEs obtained for mixture feeds could be attributed to increased starch-protein interactions. The starch-protein interactions favor the formation of insoluble polymers that reduce the water holding capacity of both starch and protein (Allen et al., 2007), thereby providing more water for lubricating screws and the barrel wall. Furthermore, proteins affect expansion by extensive networking through covalent and nonbonding interactions during extrusion (Moraru and Kokini, 2003).

A higher FSI for the pellets of 100% cereal grains than SBM and mixtures agrees with Larsen and Raun (2018), who observed a positive correlation between WSI and starch contents, but a negative correlation between WSI and protein contents. The increase in FSI with the increase in starch content can be linked to starch gelatinization and more bindings between particles (Rolfe et al., 2000; Hardy and Barrows, 2003; Welker et al., 2018). Higher FSI in barley than maize was surprising as barley have lower starch concentration and contain higher fiber particles. Fibers, particularly insoluble fibers, are known to cause weak points in pellets produced by conventional pelleting (Thomas et al., 1998), thus decreasing pellets' water stability (Larsen and Raun, 2018). The observed effect could be attributed to lower expansion in barley than maize, giving more tightly bound particles. Besides, insoluble fiber particles can be entangled and folded between different particles or strands of fiber (Thomas et al., 1998) in the continuous starch matrix, giving

a “plywood” type structure. This may also explain why FSI recorded in the feed mixture containing barley was higher than in the feed mixture containing maize, despite having comparatively lower starch contents. Thus, fibers in extruded pellets probably have a positive effect on the fluid stability of pellets. Other possibilities are differences in properties of starch (such as the size of granules, amylose:amylopectin ratio, amylose-lipid complex, the ability of retrogradation) and protein between barley and maize (Shewry and Halford, 2002; Zhu, 2017), which can affect the integrity of the extrudate structure (Zhang et al., 2014).

An increase in RE with the decrease in screen size in the hammer mill could be attributed to an improved homogeneity of the extrusion melt with smaller particles (Arêas, 1992). In addition, smaller particles increase friction through more contact among particles and between particles and the barrel during extrusion, thereby increasing melt temperature (Desrumaux et al., 1998) and consequently increasing starch gelatinization (Rolfe et al., 2000). However, the increase in RE with the decrease in screen size was more remarkable for barley and B+SBM feeds than maize and M+SBM feeds. This could be due to the greater effect of particle size reduction of fiber particles in barley with the decreasing screen size in the hammer mill. Additionally, finer fiber fractions increase nucleation sites for water vapors, which favor expansion by increasing the number of air cells (Lue et al., 1991). Despite the increase in RE with decreased particle size, BD was higher for 2 mm than 6 mm screen size. A possible explanation could be that smaller feed particles’ intrinsic density is higher than larger particles due to a higher surface-to-volume ratio (Offer and Dixon, 2000). However, the SD of pellets did not show such a pattern with the decrease in screen size. Improved water stability with the decrease in particle size increasing starch gelatinization has been reported (Rolfe et al., 2000). However, no such effect of particle size on improving fluid stability was observed in the present study.

494 An increase in RE by increasing screw speed is in line with previous studies (Baik et al., 2004;
495 Ainsworth et al., 2007; Fallahi et al., 2013; Kirjoranta et al., 2016). This can be linked to an
496 increase in shear rate, which increases temperature and SME (Lai and Kokini, 1991), thus
497 increasing starch gelatinization (Diosady et al., 1985). This increase in RE with increasing screw
498 speed led to decreased BD and SD of pellets. A decrease in FSI for maize with increased screw
499 speed is probably due to increased expansion leading to increased porosity of pellets with loose
500 contacts among particles. In contrast, FSI was slightly improved in mixture feeds with increased
501 expansion as expressed by a weak positive correlation between these two variables (FSI and RE).
502 Since expansion was increased with decreasing particle size and increasing screw speed in mixture
503 feeds, this could be due to increased shear rate resulting in increased starch gelatinization (Lai and
504 Kokini, 1991), thereby reducing adverse effects of starch-proteins interactions and providing more
505 tight bonding between the particles. Thus, for mixture feeds, FSI can be improved by increasing
506 the shear rate. However, increasing shear rate by increasing screw speed may not improve pellet
507 quality as the degree of starch gelatinization may decrease due to a decrease in residence time (Lin
508 et al., 1997). A possible alternative could be changing screw configuration with more mixing and
509 kneading elements.

510 It has been reported that a decrease in temperature at the die significantly increases melt viscosity
511 and consequently increases torque and SME (Akdogan, 1996) and decreases expansion of the
512 extrudates (Suknark et al., 1999). In the present study, with the application of cooling at the last
513 section (close to the die) in the extruder barrel, melt viscosity was possibly increased due to
514 decreased temperature, leading to increased SME and reduced RE. Consequently, both BD and
515 SD were increased. However, reduced RE with increased SME contradicts our findings where an
516 increase in SME was related to an increase in RE. Usually, SME is positively correlated with RE

(Ainsworth et al., 2007), which agrees with our study when the temperature was not controlled by cooling in the last section. The decrease in RE with the increase in SME when cooling is applied at the last section in the extruder confirms that SME affects expansion by changing the rheological properties of extrusion melt and viscous dissipation of temperature (Kristiawan et al., 2016). Thus, by counteracting the change in viscosity and temperature as done in the present study by cooling the last section, the SME may increase due to an increase in torque but without influencing expansion. A greater decrease in RE by cooling at the last section for maize could be attributed to its continuous starch melt, which probably was easy to compact compared to other feed materials containing relatively high contents of elastic fibers. Feed material factors (e.g., high starch content and small particle size) promoting RE also resulted in a greater decrease in RE when the temperature was decreased by cooling at the last section in the extruder. Thus, the highest density pellets were obtained for maize with smaller particle size and processed at low screw speed. Due to increased compaction of particles when cooling was applied at the last section, the bonding between particles was strong. This may have prevented rumen fluid from penetrating pellets, limiting disintegration and favoring a high FSI. However, this effect of cooling on FSI was only significant for maize feeds.

5. Conclusion

Using three feed ingredients and two mixtures thereof, the study revealed that pure barley and maize could be easily processed with a little manipulation of screw speed and temperature in the last section of the extruder barrel to get pellets with required density and fluid stability for increasing probability of rumen escape in dairy cows. With the extruder settings used, optimal density for rumen escape may be obtained for SBM and mixtures of SBM and barley and SBM

and maize, but their fluid stability is too low. Thus, further investigations regarding optimal process settings are needed for SBM and mixtures containing a high SBM proportion.

Conflict of interest statement

Two authors (E. Weurding and J. van Hees) is employed by Agrifirm, and one author (M. Grøseth) is employed by Felleskjøpet Fôrutvikling. Agrifirm has forwarded a patent application based on the work. The authors declare no other conflict of interest.

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Table 1

Chemical composition and mean particle size (MPS) of feed materials used

Items	Barley	Maize	SBM[1]	B+SBM[2]	M+SBM[3]
Dry matter (DM), g/kg	870	880	884	874	882
Chemical composition, g/kg DM					
Starch[4]	588	818	88	297	413
Crude protein[5]	108	94	487	320	313
Crude fiber[6]	43	17	50	48	35
Crude fat[7]	15	36	18	15	26
MPS[8], µm					
2 mm	468 (314)	337 (274)	449 (334)	464 (328)	399 (308)
6 mm	823 (727)	568 (523)	561 (422)	692 (569)	564 (467)

[1] Calculated as it contained 90% SBM and 10% maize. The chemical composition of 100% SBM was: Starch 7g/kg DM, Protein 531 g/kg DM, Crude fiber 54 g/kg DM, Crude fat 16 g/kg DM.

[2] Calculated as it contained 50% SBM and 50% barley

[3] Calculated as it contained 50% SBM and 50% maize

[4] Determined by enzymatic hydrolysis into glucose (McCleary et al., 1994).

[5] Estimated as N x 6.25 where N was determined according to Kjeldahl-N AOAC Method 2001.11 (Thiex et al., 2002).

[6] Determined according to filter bag technique (AOCS, 1996) using Ankom^{200} Fiber Analyzer.

[7] Determined by accelerated Solvent Extraction (ASE200, Dionex Corporation, Sunnyvale, CA, USA) method.

[8] Mean particle size determined as geometric mean diameter (geometric standard deviation) based on the formula described in the ASABE (2013) for feed materials ground at 2 and 6 mm screen size in hammer mill. MPS for 100% SBM was 461 (341) µm at 2 mm and 560 (411) µm at 6 mm screen size.

Table 2

Extrusion processing data for barley, maize, and soybean meal (SBM) as individual feeds

	FM /Feed[1]	1	2	3	4	5	6	7	8	Overall[2]	
Screen size[3] (mm)		2	2	2	2	6	6	6	6		
Extrusion											
Screw speed (rpm)		210	210	300	300	210	210	300	300		
Cooling[4]		No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
T3[5] (°C)	Barley	112	108	115	108	113	103	114	105	114 ± 1	106 ± 2
	Maize	120	106	128	108	125	106	130	107	126 ± 4	107 ± 1
	SBM	120	107	126	108	120	106	124	107	122 ± 3	107 ± 1
T5[5] (°C)	Barley	110	79	112	82	108	80	108	81	109 ± 2	81 ± 1
	Maize	115	83	127	85	125	84	133	86	125 ± 7	85 ± 1
	SBM	115	85	124	81	115	85	120	84	118 ± 4	84 ± 2
DP[6] (bar)	Barley	36	40	34	37	20	25	17	20	27 ± 10	31 ± 10
	Maize	10	18	3	13	6	17	2	10	5 ± 4	14 ± 4
	SBM	28	37	26	34	30	38	29	34	28 ± 2	36 ± 2
Torque[7] (Nm)	Barley	290	340	280	328	275	326	289	313	283 ± 7	327 ± 11
	Maize	321	328	301	291	370	379	344	295	334 ± 30	323 ± 41
	SBM	342	394	314	327	346	400	327	341	332 ± 15	365 ± 37
SME[8] (Wh/kg)	Barley	65	76	82	93	56	64	80	86	71 ± 12	80 ± 13
	Maize	71	63	84	81	76	76	98	83	82 ± 12	76 ± 9
	SBM	68	77	85	92	68	80	88	94	77 ± 11	86 ± 8

[1] Feed material (FM) in the column and feed (treatment) number in the row.

[2] The values are averages (with standard deviations) of all the feeds for the respective feed material.

[3] Screen size during grinding in hammer mill.

[4] Cooling at the last section in the extruder barrel.

[5] Temperatures measured by the sensor placed in extruder barrel at each section (T3, section 3; T5, section 5).

[6] Die pressure

[7] Engine load, maximum torque is 435 Nm.

[8] Specific mechanical energy.

Table 3

Extrusion processing data for 50:50 mixtures of barley and soybean meal (B+SBM) and maize and SBM (M+SBM)

	FM /Feed[1]	1	2	3	4	5	6	7	8	Overall[2]	
Screen size[3] (mm)		2	2	2	2	6	6	6	6		
Extrusion											
Screw speed (rpm)		210	210	300	300	210	210	300	300		
Cooling[4]		No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
T3[5] (°C)	B+SBM	108	105	114	107	108	107	112	106	110 ± 3	106 ± 1
	M+SBM	107	104	109	105	110	110	111	108	109 ± 3	107 ± 3
T5[5] (°C)	B+SBM	103	84	110	86	102	82	108	80	106 ± 4	83 ± 3
	M+SBM	102	82	107	84	104	84	108	83	105 ± 3	83 ± 1
DP[6] (bar)	B+SBM	32	35	29	33	17	24	20	23	25 ± 7	29 ± 6
	M+SBM	26	23	18	21	15	18	15	18	19 ± 5	20 ± 2
Torque[7] (Nm)	B+SBM	299	328	269	310	264	267	254	240	272 ± 19	286 ± 40
	M+SBM	271	238	207	217	230	243	186	196	224 ± 36	224 ± 22
SME[8] (Wh/kg)	B+SBM	59	67	79	88	52	53	73	68	66 ± 12	69 ± 14
	M+SBM	56	47	57	62	46	48	53	56	53 ± 5	53 ± 7

[1] Feed material (FM) in the column and feed (treatment) number in the row.

[2] The values are averages (with standard deviations) of all the feeds for the respective feed material.

[3] Screen size during grinding in hammer mill.

[4] Cooling at the last section in the extruder barrel.

[5] Temperatures measured by the sensor placed in extruder barrel at each section (T3, section 3; T5, section 5).

[6] Die pressure

[7] Engine load, maximum torque is 435 Nm.

[8] Specific mechanical energy.

Table 4

Bulk density (BD) and sinking velocity (SV) of pellets with their standard deviations

	FM /Feed[1]	1	2	3	4	5	6	7	8
Screen size[2] (mm)		2	2	2	2	6	6	6	6
Extrusion									
Screw speed (rpm)		210	210	300	300	210	210	300	300
Cooling[3]		No	Yes	No	Yes	No	Yes	No	Yes
BD (g/L)	Barley	548 ± 4	613 ± 2	469 ± 2	565 ± 1	502 ± 2	627 ± 4	429 ± 5	561 ± 3
	Maize	400 ± 3	835 ± 1	284 ± 4	755 ± 2	360 ± 2	778 ± 1	284 ± 4	685 ± 4
	SBM	646 ± 3	698 ± 2	628 ± 2	668 ± 2	646 ± 2	697 ± 2	626 ± 6	689 ± 2
	B+SBM	626 ± 2	659 ± 5	568 ± 4	648 ± 2	600 ± 4	633 ± 3	571 ± 1	626 ± 4
	M+SBM	701 ± 1	744 ± 3	651 ± 4	689 ± 2	683 ± 6	709 ± 6	635 ± 4	720 ± 3
SV[4] (mm/sec)	Barley	34 ± 8 (90)	100 ± 13	00	41 ± 17 (70)	20 ± 11 (60)	112 ± 4	00	74 ± 18
	Maize	00	180 ± 8	00	124 ± 15 (85)	00	128 ± 8	00	100 ± 26 (70)
	SBM	110 ± 1	112 ± 4	110 ± 1	112 ± 4	108 ± 4	114 ± 5	108 ± 4	110 ± 1
	B+SBM	100 ± 8	114 ± 6	69 ± 7 (60)	107 ± 10	109 ± 8	114 ± 5	70 ± 12	110 ± 2
	M+SBM	130 ± 10	156 ± 13	104 ± 9 (90)	127 ± 6	107 ± 11	116 ± 5	100 ± 12 (80)	119 ± 16

[1] Feed material (FM) in the column and feed (treatment) number in the row.

[2] Screen size during grinding in hammer mill.

[3] Cooling at the last section in the extruder barrel.

[4] Numbers in the parenthesis represent percentages of sinking pellets measured up to 20 min after dropping the pellet. (00) represent floating pellets.

Table 5

Specific density (SD) of pellets and specific density of pellets in rumen fluid (SD_{rf})[1] with their standard deviations

	FM /Feed[2]	1	2	3	4	5	6	7	8
Screen size[3] (mm)		2	2	2	2	6	6	6	6
Extrusion									
Screw speed (rpm)		210	210	300	300	210	210	300	300
Cooling[4]		No	Yes	No	Yes	No	Yes	No	Yes
SD (g/mL)	Barley	0.89 ± 0.02	0.99 ± 0.02	0.76 ± 0.01	0.89 ± 0.01	0.85 ± 0.01	1.10 ± 0.01	0.78 ± 0.02	1.03 ± 0.06
	Maize	0.64 ± 0.02	1.23 ± 0.02	0.44 ± 0.01	1.10 ± 0.02	0.57 ± 0.01	1.18 ± 0.02	0.43 ± 0.02	1.07 ± 0.14
	SBM	0.98 ± 0.01	0.97 ± 0.06	0.94 ± 0.04	1.02 ± 0.06	0.98 ± 0.06	1.03 ± 0.03	1.00 ± 0.02	1.04 ± 0.02
	B+SBM	0.99 ± 0.07	1.02 ± 0.04	0.89 ± 0.07	1.04 ± 0.05	0.97 ± 0.06	1.08 ± 0.03	0.96 ± 0.03	1.07 ± 0.03
	M+SBM	1.09 ± 0.03	1.18 ± 0.03	1.08 ± 0.04	1.10 ± 0.01	1.08 ± 0.03	1.09 ± 0.02	1.00 ± 0.05	1.12 ± 0.05
SD_{rf} (g/mL)	Barley	1.01 ± 0.01	1.11 ± 0.01	0.99 ± 0.01	1.08 ± 0.02	1.03 ± 0.04	1.20 ± 0.04	0.98 ± 0.01	1.13 ± 0.02
	Maize	0.83 ± 0.01	1.34 ± 0.03	0.74 ± 0.01	1.11 ± 0.02	0.77 ± 0.01	1.20 ± 0.01	0.67 ± 0.01	1.12 ± 0.14
	SBM[5]	-	-	-	-	-	-	-	-
	B+SBM	1.10 ± 0.02	1.12 ± 0.01	0.99 ± 0.01	1.09 ± 0.02	1.11 ± 0.01	1.16 ± 0.01	1.08 ± 0.02	1.14 ± 0.02
	M+SBM	1.12 ± 0.01	1.22 ± 0.09	1.12 ± 0.01	1.14 ± 0.02	1.09 ± 0.03	1.12 ± 0.03	1.07 ± 0.03	1.15 ± 0.01

[1] SD of pellets after soaking in rumen fluid at 39°C for 20 min.

[2] Feed material (FM) in the column and feed number (treatment) in the row.

[3] Screen size during grinding in hammer mill.

[4] Cooling at the last section in the extruder barrel.

[5] Not possible due to quick pellet disintegration.

Table 6

P values for main effects and interaction effects of feed material (FM), screen size in hammer mill (SH), screw speed (SS), and cooling (C) at the last section in the extruder barrel on physical properties[1] of pellets for individual ingredient feeds (first trial)

Item	RE	BD	SV	SD	FSI
FM	<.001	0.001	0.003	0.018	<.001
SH	0.001	0.038	NS	NS	NS
SS	<.001	0.002	0.020	0.020	NS
C	<.001	<.001	0.001	0.001	0.038
FM x SH	0.002	0.085	NS	NS	NS
FM x SS	0.002	0.024	0.097	0.064	0.085
FM x C	<.001	<.001	0.004	0.002	0.074
SH x SS	0.017	NS	NS	NS	NS
SH x C	NS	NS	NS	0.090	NS
SS x C	NS	NS	0.088	NS	NS
FM x SH x SS	0.099	NS	NS	NS	NS
FM x SH x C	NS	0.082	NS	NS	NS
FM x SS x C	NS	NS	NS	NS	0.069

[1] Radial expansion = RE, Bulk density = BD, Sinking velocity = SV, Specific density = SD, Fluid stability index = FSI.

765 **Table 7**

766 LS means of the physical properties[1] of pellets for the individual ingredient feeds (first trial) by
767 main effects of feed material (FM), screen size in hammer mill (SH), screw speed (SS), and
768 cooling (C) at the last section in the extruder barrel

Parameters	Levels	RE (%)	BD (g/L)	SV (mm/sec)	SD (g/mL)	FSI (%)
FM	Barley	30^{b}	539^{b}	48^{b}	0.91^{b}	92^{a}
	Maize	59^{a}	548^{b}	63^{b}	0.83^{c}	87^{b}
	SBM	9^{c}	662^{a}	111^{a}	1.00^{a}	8^{c}
	SEM[2]	0.6	4.5	4	0.01	1.0
SH	2 mm	37^{a}	592^{a}	75	0.89	63
	6 mm	29^{b}	574^{b}	73	0.93	62
	SEM[3]	0.5	3.7	3	0.01	0.8
SS	210	28^{b}	612^{a}	85^{b}	0.95^{a}	62
	300	37^{a}	554^{b}	63^{a}	0.87^{b}	62
	SEM[3]	0.5	3.7	3	0.01	0.8
C	No	45^{a}	485^{b}	41^{b}	0.77^{b}	60^{b}
	Yes	20^{b}	681^{a}	107^{a}	1.05^{a}	64^{a}
	SEM[3]	0.5	3.7	3	1.01	0.8

769 [1] Radial expansion = RE, Bulk density = BD, Sinking velocity = SV, Specific density = SD, Fluid stability index =
770 FSI.

771 [2] Standard error of the mean (n = 8)

772 [3] Standard error of the mean (n = 12)

773 [a, b, c] Indicate significant differences among the levels of a specific independent variable for a given dependent
774 variable.

Table 8

LS means of the physical properties[1] of pellets for the individual ingredient feeds (first trial) by two-way interactions between feed material and screen size in hammer mill (SH), screw speed (SS), and cooling (C) at the last section in the extruder barrel

Parameters	Levels	Feed materials	RE (%)	BD (g/L)	SV (mm/sec)	SD (g/mL)	FSI (%)
SH	2 mm	Barley	40^{c}	549^{b}	44^{c}	0.89	90
		Maize	62^{a}	568^{b}	77^{b}	0.81	89
		SBM	9^{e}	660^{a}	111^{a}	0.98	8
	6 mm	Barley	20^{d}	530^{c}	53^{bc}	0.94	93
		Maize	57^{b}	527^{c}	56^{bc}	0.84	84
		SBM	9^{e}	664^{a}	110^{a}	1.0	9
		SEM[2]	0.8	6.4	6	0.02	1.4
SS	210 rpm	Barley	27^{d}	573^{b}	68^{b}	0.96^{ab}	91^{a}
		Maize	48^{b}	593^{b}	76^{b}	0.91^{ab}	90^{a}
		SBM	10^{e}	672^{a}	111^{a}	0.99^{a}	6^{c}
	300 rpm	Barley	33^{c}	506^{c}	29^{c}	0.87^{b}	92^{a}
		Maize	70^{a}	502^{c}	50^{bc}	0.74^{c}	84^{b}
		SBM	8^{e}	653^{a}	110^{a}	1.00^{a}	10^{c}
		SEM[2]	0.8	6.4	6	0.02	1.4
C	No	Barley	36^{b}	487^{e}	15^{c}	0.82^{c}	92^{a}
		Maize	91^{a}	332^{f}	00^{c}	0.52^{d}	81^{b}
		SBM	9^{e}	636^{c}	109^{ab}	0.97^{b}	7^{c}
	Yes	Barely	24^{d}	592^{d}	81^{b}	1.00^{b}	92^{a}
		Maize	28^{c}	763^{a}	126^{a}	1.14^{a}	92^{a}
		SBM	9^{e}	688^{b}	112^{ab}	1.02^{b}	9^{c}
		SEM[2]	0.8	6.4	6	0.02	1.4

[1] Radial expansion = RE, Bulk density = BD, Sinking velocity = SV, Specific density = SD, Fluid stability index = FSI.

[2] Standard error of the mean (n =4)

[a, b, c, d, e] Indicate significant differences among the levels of a specific independent variable relative to the feed material for a given dependent variable. Differences due to main effects were not mentioned when the corresponding interaction effect was not significant.

785 **Table 9**

786 *P* values for main effects and interaction effects of feed material (FM), screen size in hammer mill
787 (SH), screw speed (SS), and cooling (C) at the last section in the extruder barrel on physical
788 properties[1] of pellets for mixture feeds (second trial)

Item	RE	BD	SV	SD	FSI
FM	0.043	0.010	0.021	0.083	0.090
SH	0.018	NS	0.050	NS	0.083
SS	0.020	0.066	0.015	NS	NS
C	0.007	0.026	0.012	NS	NS
FM x SH	0.027	NS	0.037	NS	NS
FM x SS	0.060	NS	NS	NS	NS
FM x C	NS	NS	NS	NS	NS
SH x SS	0.099	NS	NS	NS	NS
SH x C	NS	NS	NS	NS	NS
SS x C	0.049	NS	0.043	NS	NS
FM x SH x SS	NS	NS	NS	NS	NS
FM x SH x C	NS	NS	NS	NS	NS
FM x SS x C	NS	NS	NS	NS	NS

789 [1] Radial expansion = RE, Bulk density = BD, Sinking velocity = SV, Specific density = SD, Fluid stability index =
790 FSI.

Table 10

LS means of the physical properties[1] of pellets for mixture feeds (second trial) by main effects of feed material (FM), screen size in hammer mill (SH), screw speed (SS), and cooling (C) at the last section in the extruder barrel

Parameters	Levels	RE (%)	BD (g/L)	SV (mm/sec)	SD (g/mL)	FSI (%)
FM	B+SBM	9.4[a]	616[b]	99[b]	1.00[b]	27[a]
	M+SBM	7.3[b]	692[a]	117[a]	1.09[a]	17[b]
	SEM[2]	0.3	6	2	0.02	2.4
SH	2 mm	10.0[a]	661	114[a]	1.05	16[b]
	6 mm	6.7[b]	648	103[b]	1.04	27[a]
	SEM[2]	0.3	6	2	0.02	2.4
SS	210	6.8[b]	669[a]	119[a]	1.06	18
	300	9.9[a]	640[b]	98[b]	1.03	25
	SEM[2]	0.3	6	2	0.02	2.4
C	No	11.0[a]	631[b]	96[b]	1.01	26
	Yes	5.7[b]	678[a]	120[a]	1.08	18
	SEM[2]	0.3	6	2	0.02	2.4

[1] Radial expansion = RE, Bulk density = BD, Sinking velocity = SV, Specific density = SD, Fluid stability index = FSI.

[2] Standard error of the mean (n = 8)

[a, b] Indicate significant differences among the levels of a specific independent variable for a given dependent variable.

Table 11

LS means of the physical properties[1] of pellets for mixture feeds (second trial) by two-way interactions between feed material and screen size in hammer mill (SH), screw speed (SS), and cooling (C) at the last section in the extruder barrel

Parameters	Levels	Feed materials	RE (%)	BD (g/L)	SV (mm/sec)	SD (g/mL)	FSI (%)
SH	2 mm	B+SBM	12.4[a]	625	98[b]	0.98	22
		M+SBM	7.6[b]	696	129[a]	1.11	11
	6 mm	B+SBM	6.4[b]	607	101[b]	1.02	32
		M+SBM	7.0[b]	688	105[b]	1.07	23
		SEM[2]	0.4	8	3	0.03	3.3
SS	210 rpm	B+SBM	7.0[b]	629	109	1.01	23
		M+SBM	6.6[b]	707	129	1.11	14
	300 rpm	B+SBM	11.8[a]	603	89	0.99	31
		M+SBM	8.0[b]	677	106	1.07	20
		SEM[2]	0.4	8	3	0.03	3.3
C	No	B+SBM	11.7	591	87	0.95	28
		M+SBM	10.4	671	105	1.06	24
	Yes	B+SBM	7.2	642	111	1.05	26
		M+SBM	4.2	714	129	1.12	10
		SEM[2]	0.4	8	3	0.03	3.3

[1] Radial expansion = RE, Bulk density = BD, Sinking velocity = SV, Specific density = SD, Fluid stability index = FSI.

[2] Standard error of the mean (n =4)

[a, b] Indicate significant differences among the levels of a specific independent variable relative to the feed material for a given dependent variable. Differences due to main effects were not mentioned when the corresponding interaction effect was not significant.

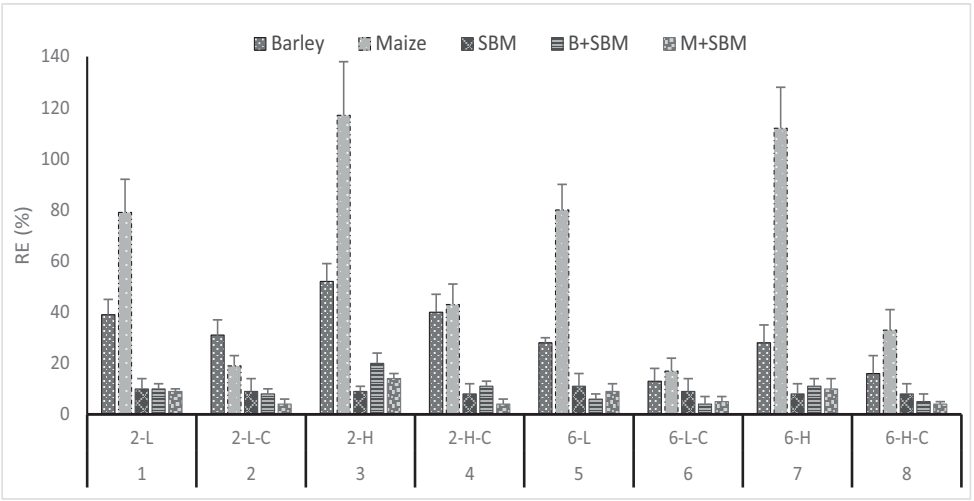
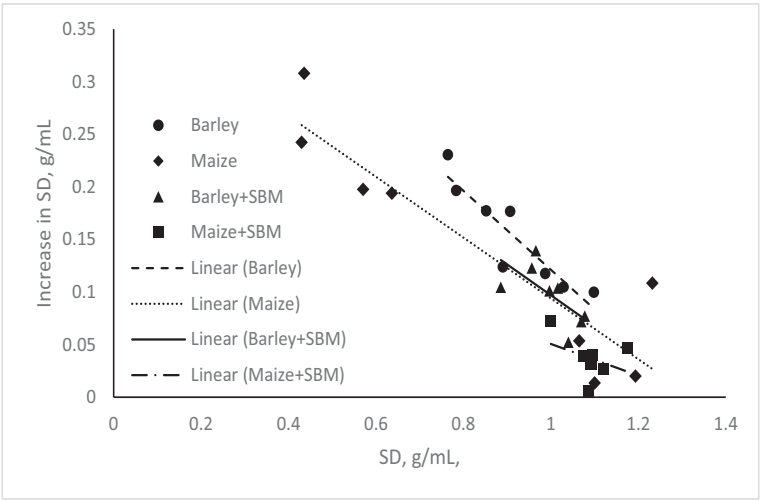


Figure 1. Radial Expansion (RE) of feed pellets (bars as standard deviations of thirty measurements). At horizontal axes, upper row 2 and 6 represent grinding in hammer mill with either 2 mm or 6 mm screen size, respectively, L is for low screw speed (210 rpm), H is for high screw speed (300 rpm), and C is for cooling at the last section in the extruder. Lower row, numbers 1-8 represent feed or treatment number.



815

816 **Figure 2.** Relationship of specific density (SD) with the increase in SD of pellets after soaking for

817 20 min in rumen fluid at 39 ℃.

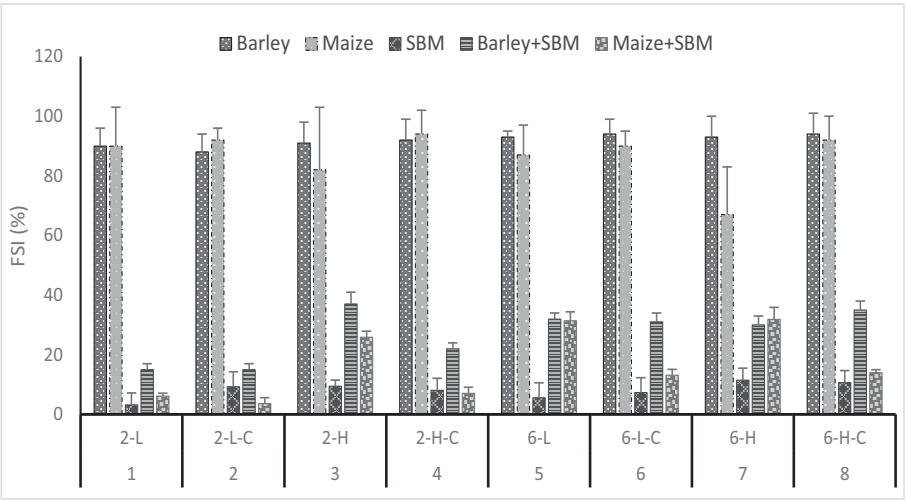


Figure 3. Fluid stability index (FSI) of feed pellets (bars as standard deviations of three measurements). At horizontal axes, upper row 2 and 6 represent grinding in hammer mill with either 2 mm or 6 mm screen size, respectively, L is for low screw speed (210 rpm), H is for high screw speed (300 rpm), and C is for cooling at the last section in the extruder. Lower row, numbers 1-8 represent feed number.

Paper-II

Effects of density of extruded pellets on starch digestion kinetics, rumen fermentation, fiber digestibility, and enteric methane production in dairy cows

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Abstract

The kinetics of starch utilization in dairy cows fed extruded pellets differing in physical functional properties was investigated by measuring starch digestibility, postprandial rumen fermentation patterns, and postprandial duodenal starch appearance. Additionally, starch digestion effects on neutral detergent fiber (NDF) digestibility and methane (CH_4) emission were studied. Pure barley was used during extrusion to produce three treatments having pellets of either low-density (LD), medium-density (MD), or high-density (HD). The experiment was conducted in a 3×3 Latin square design using three lactating Danish Holstein cows fitted with ruminal, duodenal, and ileal cannulas. Due to problems with involuntary intake, all treatments were fed directly into the rumen through the rumen cannula to simulate pellets' entrance into the reticulo-rumen by eating. After the allocation of experimental concentrate, cows were fed a basal diet low in starch. Eight samples were collected on equal time intervals (9 hours) from duodenal digesta, ileal digesta, and feces (grab sample) to determine digestibility. For postprandial rumen fermentation patterns, four sample sets of rumen dorsal, medial, and ventral fluid were taken from each cow at 2, 4, 6, 8 h, whereas for postprandial duodenal starch appearance, samples of duodenal chyme were obtained from each cow at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16 h relative to morning feeding of the experimental concentrate at 07:00 h. The ruminal, small intestinal, hindgut, and total tract digestibility of starch did not differ among the treatments and were on average 82 ± 4%, 97 ± 0.8%, and 99 ± 0.1%, respectively. Similarly, NDF digestibility and CH_4 emission also remained unaffected by the treatments. However, HD treatment showed higher acetate:propionate ratio at all positions in the rumen and a higher postprandial duodenal starch appearance than LD and MD treatments. This indicates lower ruminal starch digestion (RSD) for HD treatment and a higher starch flow into the small intestine where mostly digested

and absorbed. In conclusion, the current study indicates that pellets' physical properties can manipulate RSD, where pellets with high density and fluid stability can partly shift starch digestion from the rumen to the small intestine. Indeed, further investigations are needed.

Abbreviations: Ac:Pc, acetate:propionate ratio; CP, crude protein; DM, dry matter; DMI, dry matter intake; FSI, fluid stability index; HD, high density; LD, low density; MD, medium density; aNDFom, neutral detergent fiber; PMR, partial mixed ration; RPM, rotations per min; RSD, ruminal starch digestibility; SISD, small intestine starch digestion; TTSD, total tract starch digestion; VFA, volatile fatty acids

Keywords: In vivo; Extrusion; Ruminal degradation; Starch

1. Introduction

High-producing dairy cows have high demands for the supply of energy, especially in early lactation. In that period, feed intake, despite increasing, cannot match milk production demands (Allen et al., 2005). Usually, the need for energy is met by increasing the amount of concentrate fed. However, high levels of rapidly fermentable starch from concentrates may negatively affect the rumen environment and increase the risk of rumen acidosis (Owens et al., 1998). It has been demonstrated that the energy efficiency is about 42% higher for small intestinal starch digestion compared to starch digested in the rumen (Brake and Swanson, 2018). Thus, partly shifting the starch digestion site from the rumen to the small intestine could optimize feed intake and feed utilization in high producing ruminants. Most commonly, the site of nutrient digestion is shifted by altering the rate of rumen digestion either by the selection of feed ingredients or application of various feed processing methods. However, since rumen digestion results from the concurrent rate of ruminal degradation and rate of passage, increasing the rate of passage, especially if combined with a lower rate of degradation, will result in the shift of digestion site from the rumen to the small intestine. Therefore, manipulating passage kinetics also could be an alternative approach to alter the site of nutrient digestion.

Although intrinsic physical properties of feed particles influencing their outflow from reticulorumen were identified a long time ago (King and Moore, 1957; Campling and Freer, 1962), altering digestion site by manipulating the passage rate of feed particles is scarcely studied. Based on studies using either inert plastic particles or labeled indigestible plant fiber particles, it is revealed that the rate of passage of rumen particulate matter is mainly dependent upon particle density (Lechner-Doll et al., 1991). High density (sinking) particles have a higher passage rate from reticulorumen than particles with low density (floating). However, controlling the density of

feed particles during processing is not easy. Since concentrate feedstuffs are being increasingly pelletized by conventional pelleting to ease on-farm allocation, feed pellets with some specific density may be used to manipulate the passage rate and thereby alter the site of nutrient digestion in ruminants. Conventional pellets exhibit high density and have low water stability (Larsen and Raun, 2018) and therefore may disintegrate rapidly in the rumen, losing their physical properties. Extrusion feed processing is being extensively used in the fish feed industry to obtain compound feed pellets with functional physical properties like density with varying sinking velocities in seawater and high water stability (Sørensen, 2012; Welker et al., 2018). Recently, the effect of extruded pellets' physical properties on rumen environment variables and postprandial starch appearance in the duodenum was studied *in vivo* (Larsen et al., 2019). They compared conventional pellets with extruded pellets of wheat, maize, and mixtures of them and soybean meal having either low-density or high-density based on their bulk densities. They could not observe any apparent effect of treatments on the variables studied. However, they suggested that feed pellets with high density and high liquid stability could increase postprandial duodenal flow that may influence a nutrient's rumen digestion kinetics. By analyzing the behavior of extruded pellets in rumen fluid during *in vitro* study, Khan et al. (2021) demonstrated that specific density did not correlate with bulk density for high-density feed pellets. Thus, determination of specific density is important when feed pellets are intended for increased passage from reticulorumen.

The present study's objective was to investigate the effects of pellets' physical properties on starch digestion kinetics along the gastrointestinal tract, rumen fermentation patterns, postprandial duodenal starch flow, fiber digestion, and methane emission by using extruded barley. We hypothesized that the high-density (or high sinking velocity) pellets would increase the passage rate, resulting in less rumen digestion and more rumen escape of starch.

2. Materials and Methods

The present experiment complied with Danish Ministry of Justice Law no. 382 (June 10, 1987), Act no. 726 (September 9, 1993), concerning experiments with animals and experimental animals' care.

2.1. Processing of Experimental Treatments

The experimental concentrate was composed of pure barley grain and was processed at Fôrtek (Center for Feed Technology), NMBU, Ås, Norway, to obtain three treatments. The barley was ground using a hammer mill equipped with a 6 mm screen (HM 21.115, Münch-Wuppertal, Germany), and the meal was subsequently divided into three portions. All portions of the meal were preconditioned in a double shaft conditioner (BCTC 10, Bülher, Uzwil, Switzerland) and then extruded (Twin Screw BCTG 62 Extruder, Bülher, Uzwil, Switzerland) using a 6 mm die. Three different extruder processing settings to get pellets of either low, medium, or high density. Low-density (LD) treatment was produced using high screw speed (300 rpm), giving an exit temperature of 120 °C, which resulted in extrudate expansion. Medium-density (MD) treatment was obtained by reducing the extent of expansion with low screw speed (210 rpm), giving an exit temperature of 113 °C. High-density (HD) treatment was produced by limiting the extrudate expansion using low screw speed (210 rpm) with cooling of the last section of the extruder barrel, intended to keep the exit temperature around 90 °C as maximum. The maximum temperatures obtained during processing were 122, 116, 109 °C for LD, MD, and HD treatments, respectively. The extruded pellets were dried in a fluid bed continuous dryer (Fôrtek, NMBU) at ~100 °C with a retention time of 7-10 min and afterward cooled at room temperature. When steady-state processing conditions were achieved, a sample was taken for each treatment at the start, the middle,

the end of production. These three samples were pooled into one sample for each treatment and used to analyze physical properties.

2.2. Analysis of Physical Properties

Bulk densities were determined in triplicate as described by Sørensen (2012), in which the weight of pellets was measured using a 1 L steel cylinder. Radial expansion of pellets was calculated as a ratio of a pellet's diameter, measured with an electronic sliding caliper, to the diameter of the die and expressed in percentage. The reported value is the average of 30 measurements. Hardness was determined on a texture analyzer HK5T (Tinius Olsen Ltd., UK) fitted with a 100 N load cell using a flat knob and 10 mm/min compression speed. The force (N) used to make the first crack in the pellet was used as a hardness value. Each reported value is the average of 30 measurements. Specific density, sinking velocity, and fluid stability index (FSI) were performed as described in Khan et al. (2021). In short, specific density was determined by measuring the weight of five selected pellets and then the pellets' volume by volumetric displacement method using 0.5 mm glass beads in tapped density analyzer (AUTOTAP, Quantachrome Instruments, 1900 Corporate Drive, Boynton Beach, Florida, USA). Each reported value is the average of five measurements. Sinking velocity test was performed by measuring the time taken by a pellet to pass a distance of 220 mm in a transparent glass cylinder (310 mm high and 35 mm inner diameter), filled with rumen fluid of approximately 39 °C. Each value is the average of 30 pellets measurements. The FSI of pellets was determined in triplicate by measuring the dry matter remained in 2 mm mesh net ball-shaped baskets after incubation in rumen fluid at 39 °C for 30, 60, and 120 min.

2.3. Animal Experiment

The three treatments were tested in a 3 × 3 Latin square experiment with 21-day periods having 11 days of adaptation and ten days of sampling. Three lactating Danish Holstein cows (weighing 700 ± 52 kg, 253 ± 146 days in milk, and yielding 33 ± 5 kg milk/d) fitted with ruminal (#1C; Bar Diamond, Inc., Parma, ID, USA), duodenal (open T-piece placed 60 cm caudal to the pylorus), and ileal (open T-piece placed 20 cm cranial to the caecum) cannulas were used. Cows were housed in tie stalls with mattresses and had free access to water. A total of 4.8 kg/d of each treatment was fed in two equally divided portions at 7:00 and 16:30. Due to low palatability observed during pre-trial testing, all treatments were fed directly via the rumen cannula. To simulate the entrance into the reticulo-rumen by eating, pellets were emptied from small plastic bags for 10 min as close as possible to the esophageal opening. The external digesta flow marker (13 g of TiO_2) was placed into the rumen dorsal sac at the end of concentrate feeding. Thirty min after concentrate feedings, a PMR (Table 2) was allocated *ad libitum* with 60% of daily allowance in the morning. Residual PMR was removed and weighed just before morning milking. Cows were milked at 06:00 and 16:00, and milk volume was recorded each time.

Samples for the determination of nutrient digestibility were collected from day 12 to 15 in each period. The samples of concentrate, PMR, and residual PMR were subsampled for dry matter (DM) determination and subsequently stored frozen at -20 °C until preparation for chemical analysis. PMR samples were pooled within the period, whereas one sample of each concentrate was taken in each period. Eight samples were collected on equal time intervals (on day 12 at 18:00; day 13 at 0:300, 12:00, 21:00; day 14 at 06:00, 15:00, 24:00; day 15 at 09:00) from duodenal digesta (500 ml), ileal digesta (300 ml) and feces (~250 ml grab sample) and pooled within cow and period. The duodenal and ileal samples were collected in tube-shaped plastic bags mounted to the cannula

with plastic knees. The pooled samples of digesta and feces were stored frozen at -20 ℃ until preparation for chemical analysis.

To determine the diurnal rumen fermentation pattern, eight samples of rumen fluid were taken from the ventral rumen at time-points corresponding to digesta samplings. For the determination of the postprandial fermentation pattern, rumen fluid samples were taken on day 15. Both diurnal and postprandial samples were drawn with the same procedure using a suction strainer (#RT, Bar Diamond Inc.) equipped with a 50 ml syringe. For postprandial samples, rumen fluid was taken from dorsal, medial, and ventral rumen at 2, 4, 6, 8 h relative to the feeding of the experimental pellets at 07:00. At first, about 40 ml of rumen fluid was sucked into a 50 ml syringe from the rumen's ventral sac and transferred to a 50 ml Falcon tube. Then the strainer was pulled upward about 25-30 cm to get a sample from the medial rumen. The sample from the dorsal rumen was taken through or just below the upper fiber mat. The pH in rumen fluid samples was measured immediately using a combination electrode (PHC2002–8, Hach Lange ApS, Brønshøj, Denmark) and a pH meter (PHM240 pH/ION Meter, MeterLab, Radiometer analytical, Copenhagen, Denmark) calibrated at pH 4.000 and 7.000. Each rumen fluid sample was then subsampled into three Sarstedt tubes (10 mL in each) and stored at −20 °C until the analysis of volatile fatty acids (VFA).

For postprandial duodenal starch flow, Cr-EDTA was used as digesta flow marker as follows: 22 h before first sampling, a priming dose of 400 mL Cr-EDTA infusate (3.1 ± 0.06 g Cr/L) was administered to the ventral rumen followed by continuous infusion at a rate of 60 ± 3 mL/h using a peristaltic pump. On day 19, samples of duodenal chyme were obtained from each cow at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16 h relative to morning feeding of concentrate at 07:00. Samples (200 mL) were immediately transferred to plastic containers and weighed. About 13 mL of fluid

from each sample was transferred to a Sarstedt tube and centrifuged to extract liquid. 8 mL of supernatant was transferred to another tube and stored at −20 °C for Cr determination. The remaining supernatant and precipitate were transferred back into the respective sample. The duodenal digesta samples were then dried at 100 °C in a forced air oven for 48 h, DM was determined, and the dried samples were stored for later grinding and starch analysis.

On days 20 and 21, the exchange of CH_4, CO_2, and H_2 was measured in respiration chambers. The system and equipment are described in detail by Hellwing et al. (2012). In the two first periods, there were technical problems with the CH_4 sensor (Horiba VIA-510 infrared CH4 sensor (Horiba LTD, Kyoto, Japan)). Therefore, the CH_4 emission in all three periods was measured by an infrared sensor from Guardian Range (Edinburgh sensors, Livingston, UK). Methane in period 3 was measured with both the Guardian Range sensor and Horiba CH_4 sensor. The recovery for the Horiba sensor is usually between 99-100%. The Guardian Range sensor produced lower numbers compared with the Horiba sensor, and the Guardian numbers were corrected to the same level as the Horiba sensor for all three periods. In the experiment, cow and chamber were confounded. Recovery tests for the CO_2 and CH_4 (Measured with Horiba VIA-510) were performed after the experiment and averaged 99.4 ± 1.1% and 99.0 ± 0.6% for CH_4 and CO_2, respectively. Reported numbers are corrected using these recovery factors. Hydrogen was corrected with 99.2 ± 0.83%, which is an average of the recovery for CH_4 and CO_2. Feeding and milking were done similarly as before. Samples of PMR and PMR residues were taken for dry matter determination. Gas data are reported at standard temperature and pressure (0 °C (273.15 K) and 101.325 kPa).

2.4. Chemical Analysis

The DM content of concentrates, PMR, and PMR residues was determined by drying for 48 h at 60 °C in a forced-air oven. Dried samples were subsequently ground using a Retch cutter mill with

158 a 1 mm screen to analyze nutrients except for starch analysis, where a 0.5 mm screen was used.
159 Starch content in concentrates, PMR, duodenal digesta, ileal digesta, and feces was determined
160 enzymatically (Kristensen et al., 2007) using the immobilized glucose oxidase electrode technique
161 for glucose measurements (Mason, 1983). Starch was partially hydrolyzed at 100 °C with
162 thermostable α-amylase, followed by complete hydrolysis with amyloglucosidase at 60 °C and
163 released glucose was determined by YSI 2900D analyzer (YSI Inc., Yellow Springs, Ohio, USA).
164 Starch content was corrected for the content of free glucose in the original sample by incubation
165 without enzymes. Nitrogen content in concentrates, PMR, duodenal digesta, ileal digesta, and feces
166 was determined according to the Dumas method (Hansen, 1989) by using a Vario Max CN
167 (Elementar Analysesysteme GmbH, Hanau, Germany). Neutral detergent fiber (NDF) in
168 concentrates, PMR, duodenal digesta, ileal digesta, and feces was determined using Fibertec 2010
169 (Foss, Hillerød, Denmark) equipment after treatment with a heat-stable amylase and corrected for
170 residual ash (Mertens, 2002) and expressed as aNDFom. Ash content in concentrates and PMR
171 was determined by combustion for 6 h at 525 °C (method 923.03;(AOAC, 1990)). The TiO_2
172 content in PMR, duodenal digesta, ileal digesta, and feces was analyzed as described by Myers et
173 al. (2004). The Cr in the supernatant (from duodenal flow samples) was analyzed by atomic
174 absorption spectroscopy at 357.9 nm, as described by (Williams et al., 1962). Concentrations of
175 VFA and L-lactate were analyzed by gas chromatography (Kristensen et al., 1996) and
176 immobilized L-lactate oxidase electrode technique (YSI 2900D, YSI Inc., Yellow Springs),
177 respectively, after stabilizing rumen fluid with 25% m-phosphoric acid (MPA)/2-EB solution.

178 *2.5. Calculations and Statistical Analysis*

179 The DM flow of duodenal and ileal digesta and fecal output was calculated from daily TiO_2 doses
180 and concentrations at their respective sites. The flow of nutrients was calculated from DM flow

and chemical analysis of DM at each site. The apparent digestibility of nutrients in each section of the gastrointestinal tract was calculated based on the inflow and outflow of nutrients at each respective section. The postprandial duodenal DM flow was calculated using Cr as an indigestible marker, assuming constant hourly rumen Cr outflow as rumen infusion was continuous. Subsequently, the postprandial duodenal starch flow was calculated from DM flow and the percentage of starch present in duodenal DM at each time point.

Feed intake, nutrient digestibility, and methane emission data were statistically analyzed using the GLM procedure in SAS (2013) and period, treatment (experimental concentrate), and cow as fixed effects in the model. The postprandial and diurnal rumen fermentation variables and postprandial duodenal DM and starch flow were statistically analyzed using the MIXED procedure of SAS (2013) for repeated measurements with the period, treatment (Trt), Time and Trt × time as fixed effects, and cow as a random factor. Time within cow × period was considered a repeated measurement using the autoregressive (AR1) covariance structure. The Kenward-Roger method was used to calculate denominator degrees. The results are reported as least square means (LS means) with standard error of the mean (SEM) for each treatment. Significance was claimed when $P \leq 0.05$ and tendencies were considered at $0.05 < P \leq 0.10$.

3. Results

3. 1. Chemical Composition and Physical Properties of Experimental Treatments

The experimental treatments did not vary in their chemical composition, except dry matter (Table 1). The length by diameter (mm) of pellets was 13 × 9, 8 × 7, and 7 × 6 for the LD, MD, and HD treatment, respectively. The anticipated differences in physical functional properties of pellets were obtained as treatments varied in their bulk densities and specific densities from 384 to 602

g/L and 0.66 to 1.04 g/mL, respectively, giving pellets with floating (LD), slow sinking (MD), and fast sinking (HD) properties. Moreover, all treatments showed higher than 85% FSI after 120 min. incubation (Table 1; Figure 1).

3. 2. Intake, Flow, and Digestibility

For concentrate, a higher DM intake (DMI) for the MD treatment was observed ($P = 0.01$; Table 3) due to higher DM content in MD treatment (Table 1) compared to the LD and HD treatments. However, total DMI was not affected by treatments ($P = 0.68$; Table 3). Intake of nutrients also did not differ among treatments ($P \geq 0.33$). Milk yield was not affected ($P = 0.17$) by treatments and averaged 23.1 ± 0.8 kg/d.

The flow of glucose and starch in the duodenum, ileum, and feces did not differ among treatments ($P \geq 0.15$; Table 4). The ruminal starch digestibility (RSD) was not affected by treatments ($P = 0.43$). Similarly, small intestinal, hindgut, and total tract digestibility of starch (TTSD) did not differ ($P \geq 0.23$) among treatments. Only a small fraction of starch escaping the small intestinal digestion was digested in the hindgut, and TTSD was 99 ± 0.1% for all treatments.

The ruminal, small intestinal, hindgut, and total tract digestibility of DM and aNDFom did not differ among treatments ($P \geq 0.28$; Table 5). However, the small intestinal digestibility of aNDFom was negative for all treatments. Ruminal and total tract digestibility of CP did not differ ($P \geq 0.24$) among treatments. However, the small intestinal digestibility of CP was higher for the HD treatment than for LD and MD treatments ($P = 0.02$).

3.3 Rumen variables

Postprandial ruminal pH and concentration of total VFA in the dorsal, medial, or ventral part of the rumen did not differ among treatments ($P_{Trt} \geq 0.32$; Table 6). However, compared to the ventral

rumen, pH was lower in the dorsal and medial rumen for all treatments, reaching the lowest value at 4 h post-feeding ($P_{Time} \leq 0.02$). The concentration of total VFA was lower in the ventral rumen than dorsal and medial rumen for all treatments. The acetate to propionate (Ac:Pr) ratio was higher ($P_{Trt} = 0.03$) for the HD treatment in the medial rumen and tended ($P_{Trt} \leq 0.08$) to be higher in the dorsal and ventral rumen compared to the LD and MD treatments. The Ac:Pr ratio increased from 2 h to 8 h after feeding ($P_{Time} \leq 0.03$) for all treatments in all sections of the rumen.

Diurnal pH and total VFA concentration in the ventral rumen were not affected by treatments ($P_{Trt} \geq 0.23$; Table 7) but were affected by the time of sampling ($P_{Time} < 0.001$). Diurnal propionate proportion was lower ($P_{Trt} = 0.04$), and Ac:Pr ratio tended to be higher ($P_{Trt} = 0.09$) for HD treatment compared with MD treatment but did not differ from LD treatment.

3.4. Postprandial duodenal flow

The DMI during the postprandial sampling day was slightly lower compared to the digestibility sampling days but did not differ among the treatments ($P_{Trt} = 0.13$; Table 8). Overall, postprandial duodenal DM flow was greater with LD and HD treatments than MD treatment ($P_{Trt} = 0.01$; Figure 2A). Concerning the first postprandial sequence, duodenal starch flow and concentration did not differ among treatments ($P_{Trt} \geq 0.14$) up to 9 h after morning feeding. However, when both postprandial sequences were taken into consideration, duodenal starch flow and concentration increased towards the evening ($P_{Time} \leq 0.02$; Figure 2B, 2C), where both postprandial duodenal starch flow and concentration were highest for HD treatment ($P_{Trt} \leq 0.05$) as compared with LD and MD treatments.

3.5. Methane measurements

246 The total daily methane emission and methane emission per kg DMI did not differ between
247 treatments (Table 9).

248 **4. Discussion**

249 Barley starch is an easily digestible starch source, with an average RSD and TTSD of about 87%
250 and 96%, respectively (Nocek and Tamminga, 1991; Moharrery et al., 2014). However, these
251 values vary greatly depending upon differences between barley varieties, amount of starch intake,
252 degree of processing, and feeding level. For current treatments, it was assumed that highly stable
253 extruded pellets differing in densities would ferment at different positions in the rumen and give
254 different patterns of duodenal starch appearance. Thus, it was hypothesized that high-density
255 pellets with fast sinking behavior combined with high fluid stability would have lower
256 fermentation in the ventral rumen compartment and would have the greatest likelihood of passing
257 out of the rumen, leading to reduced RSD. Indeed, the RSD did not differ among treatments, but
258 both the postprandial duodenal starch appearance and ruminal Ac:Pr ratio were higher for HD
259 treatment compared with LD and MD treatments indicating reduced RSD with HD treatment.

260 Experiments with plastic particles have revealed that particles with specific density between 1.1
261 to 1.42 g/mL have a lower mean retention time in the rumen (desBordes and Welch, 1984; Seyama
262 et al., 2017; Dufreneix et al., 2019) and thus would have higher passage rate from the reticulorumen
263 compared with particles having a specific density below 1.1 g/mL. However, contrary to inert
264 plastic particles, feed particles change their specific densities once they are in the rumen, where
265 particle specific density may first increase due to hydration by rumen fluid and then decrease due
266 to entrapment of fermentation gases (Wattiaux et al., 1992). Dufreneix et al. (2019) suggested that
267 a particle density close to 1.3 g/mL will take more time to decrease its density below 1.1 g/mL
268 and, therefore, will have a higher chance to leave the rumen. After soaking in rumen fluid for 30

min, the specific density of HD treatment was increased from 1.04 to 1.22 g/mL, whereas the specific density of LD and MD treatments also increased but remained below 1 g/mL (Table 1). This demonstrates that functional specific density of HD treatment after entering the rumen was in the optimal range and thus resulting in higher starch flow to the duodenum compared with LD and MD treatments. Larsen et al. (2019) fed cows with extruded pellets of pure wheat and pure maize having either LD with an average bulk density of 443 ± 15 g/L or HD with an average bulk density of 658 ± 25 g/L. Contrary to our findings, they could not observe a clear difference in rumen fermentation variables and duodenal starch appearance, despite marked differences in the bulk densities of their extruded treatments. However, they did not determine specific densities of pellets. Moreover, the difference in functional specific density of pellets within the rumen may be smaller than the difference in bulk density due to physical forces by motility, digesta, and fermentation gasses. This might explain similar effects of pellet densities on rumen fermentation variables and duodenal starch appearance as observed by Larsen et al. (2019) and in the present study for LD and MD treatments.

The overall postprandial duodenal flow of starch increased at a lower rate after feeding and has a lower mean starch appearance than observed by Larsen et al. (2019) for LD and HD treatments based on 100% wheat or maize. In their study, duodenal starch flow peaked at 2.5 h post-feeding and then followed an exponential decline. Indeed, the digesta flow markers applied differed between the two studies, but the duodenal samples' current starch concentrations also indicate lower starch flow rates. Previously, Tothi et al. (2003) observed that postprandial duodenal starch flow increased at lower rates reaching peak flow at 4-6 h post-feeding by feeding barley (ground or expander treated conventionally pelleted) as a pulse dose. However, the functional physical properties of pellets used in Tothi et al. (2003) and the current study might differ in density and

fluid stability as conventional pellets typically have high density and disintegrate quickly in liquid. Using 24 commercial pelletized concentrates, Larsen and Raun (2018) observed that water stability index (WSI) varied from 2 to 20%, whereas FSI in the present study was more than 85% after 120 min incubation. Extruded pellets used in the present study can be considered highly stable in the rumen, as rumen fluid at 39 °C instead of water at 25 °C and more vigorous agitation was used to determine FSI compared to WSI. In addition to differences in physical properties, pellets were fed in both morning and evening feeding in the current study; thus, giving two peaks of postprandial duodenal starch flow. Nevertheless, postprandial duodenal starch flow for HD treatment increased gradually as the day progressed towards evening compared to LD and MD treatments (Figure 2B, 2C). It is evident that starch outflow from reticulorumen did not follow an exponential decline. Therefore, it did not follow first-order kinetics, which is generally assumed for starch, and indicated a lag time of newly ingested starch before passage. The starch outflow for LD and MD treatments was probably delayed due to their lower densities and slow disintegration. In contrast, HD treatment took a long time to attain the necessary density and perhaps size for passage out of the reticulorumen. A large peak after evening feeding, particularly for HD pellets, suggests increased starch flow due to new starch intake combined with a pulse of undigested starch either from the rumen or from the abomasum (Tothi et al., 2003). It can be speculated that duodenal starch flow was highest towards the evening, where few samples were taken, giving lower mean starch flow than observed by Larsen et al. (2019).

A 6 mm die size was used in the present study compared to Larsen et al. (2019), where extruded pellets were produced using a 2.4 mm die size. Therefore, pellets' size also differs between the two studies, which could influence passage from the reticulorumen (Offer and Dixon, 2000). Based on wet sieving analysis of digesta particles leaving the reticulorumen, the probability of passage is

315 negatively related to particle size (Poncet, 1991). However, experiments using inert plastic
316 particles have provided inconclusive results on the relationship between particle size and passage
317 kinetics. Kaske et al. (1992), using plastic particles with different lengths (1, 5, 10, and 20 mm),
318 observed a decrease in particle passage as the particle size increased; however, they demonstrated
319 that particles with 10 mm size could substantially pass from reticulorumen. Seyama et al. (2017)
320 observed a higher passage rate for spherical particles with diameters 6.35 and 7.95 mm than
321 particles with a diameter of 3.97 mm. In contrast, Dufreneix et al. (2019) recently suggested that
322 particles with a diameter between 3-4 mm would have higher flow out of the reticulorumen than
323 particles having other sizes. Nevertheless, higher duodenal starch flow rates observed by Larsen
324 et al. (2019) compared to the current study can be attributed to the smaller pellet size used in their
325 study. Despite that the way pellets were fed to the animals differed between the two studies,
326 experiments with plastic particles, administered orally or directly into the cows' rumen, revealed
327 similar effects regarding passage kinetics and rates independent of administration method
328 (desBordes and Welch, 1984). Thus, using a smaller die size (e.g., 3 mm) will give pellets that
329 could probably give higher duodenal starch flow unless the optimal density is maintained.

330 The increased HD treatment density was obtained by decreasing expansion, giving increased
331 compaction of particles and high hardness. The increased particle compaction limited the microbial
332 penetration and consequently degradation of pellets as also supported by relatively a high FSI after
333 120 min incubation for HD treatment. Contrastingly, LD and MD treatments have either high
334 expansion or low hardness; thus, making starch more susceptible to microbial breakdown
335 (Huntington, 1997; Giuberti et al., 2014). As the proportion of propionate increases relative to
336 acetate's proportion with the increase in starch digestion in the rumen (Sjaastad et al., 2016), the
337 Ac:Pr ratio decreased. This trend is evident by comparing the postprandial rumen Ac:Pr ratio with

the corresponding first postprandial sequence of duodenal starch flow. During this period, duodenal starch flow did not differ among treatments, but Ac:Pr ratio was higher for cows fed HD treatment than cows fed LD and MD treatments, indicating lower starch digestion for HD treatment. Thus, high density and high fluid stability, like in current HD treatment, are essential pellet properties resulting in higher rumen escape of starch.

It is vital that starch escaping the reticulorumen is digested in the small intestine and absorbed as glucose to achieve the actual energetic potential of shifting the starch digestion site (Huntington et al., 2006; Mills et al., 2017). Small intestinal starch digestibility (SISD) remained unaffected among the treatments. However, it seemed that SISD followed the same pattern as RSD, especially for LD and HD treatments, i.e., LD treatment with a numerically higher RSD have numerically higher SISD and vice versa. This agrees with Larsen et al. (2009) suggested that both RSD and SISD are affected by similar processes. However, on average, 97 ± 0.8% of starch intake was digested up to distal ileum, and free glucose in ileal contents did not differ among treatments. Moreover, SISD was above 80% and thereby above 75%, which is the minimum threshold value demonstrated by Huntington et al. (2006) to increase energy yield by shifting the site of starch digestion from the rumen to the small intestine. Thus, no negative impact on SISD was observed. In addition, a higher small intestinal digestibility of crude protein for HD treatment than LD and MD treatments is compelling as, based on current starch observations, a greater rumen outflow of dietary crude protein could be expected for HD treatment and needs further investigation.

An increase in RSD has been observed to be accompanied by a decrease in ruminal and total tract digestibility of fiber (McCarthy et al., 1989; Chibisa et al., 2015). Ruminal and total tract digestibility of NDF did not differ among treatments, despite an increased rumen escape of starch for HD treatment. Besides, both rumen and total tract NDF digestibility remained above 70% for

all treatments. Cellulolytic bacteria's ability to digest fiber is sensitive to pH changes, where pH below 6.0 is recognized to impair the growth of these bacteria (Van Kessel and Russell, 1996; Dijkstra et al., 2012). Despite that pH remained below 6.0 in the dorsal and medial part of the rumen, ruminal pH was not affected by the treatments, and the average ruminal pH (calculated as the average across the dorsal, medial and ventral parts of the rumen) and particularly pH in the ventral rumen was above 6.0. It can be speculated that during the current conditions, ruminal pH did not drop drastically due to an overall slow degradation of extruded pellets, thus favoring optimal ruminal conditions.

Since increased ruminal starch fermentation resulting in lower Ac:Pr ratio reduce methane emission (Mills et al., 2001), the effects of expected alterations in patterns of RSD on methane emission were also studied. A higher Ac:Pr ratio for HD treatment could be an indicator of a higher methane emission for this treatment; however, both methane emissions as L/d and L/kg DMI did not differ among treatments. The methane emission is not only affected by the fermentation pattern but also by the amount of fermented nutrients in the rumen and the use of hydrogen for other processes in the rumen. Overall, the treatments' effects had been too small to be detected in the methane data despite differences in rumen fermentation patterns.

The voluntary intake of experimental feeds was a challenge and varied among cows in pre-trail. Larsen et al. (2019) reported similar intake problems for extruded pellets, especially LD pellets of pure wheat and pure maize, but not for grain-soybean meal mixes. They observed difficulty in swallowing due to the stickiness of pellets during chewing. In the present study, the stickiness of pellets was not observed. However, the pellets used were hard and big (6-13 mm), with sharp fiber particles protruding on the pellets' edges compared to Larsen et al. (2019). It was observed that the cows were troubled in chewing while eating the pellets. Primdal et al. (2014) found that large

size (8 mm) and high hardness of pellets could decrease intake. Hence, the intake problems could be related to the size and physical shape of the extruded pellets, and the use of smaller pellets might have solved the problem for the extruded barley feeds.

5. Conclusion

The study indicated that the density of pellets could manipulate starch passage kinetics from reticulorumen. Although RSD did not differ among the treatments, the high Ac:Pr ratio and rumen escape of starch for HD treatment all point towards the support of the hypothesis that the high density combined with the high fluid stability of extruded pellets could reduce RSD by decreasing the rate of degradation and increasing the rate of passage. However, further investigations are needed with optimal pellet size and relevant composition of concentrates.

Conflict of interest statement

None of the authors have conflicts of interest

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538 **Table 1.**

539 Chemical composition and physical properties of experimental concentrate pellets

Item	Experimental concentrates pellets[1]			SEM[2]	P-value
	LD	MD	HD		
Chemical composition					
Dry matter (DM), g/kg	919[b]	928[a]	919[b]	2.2	0.04
Starch, g/kg DM	633	622	625	4.3	0.28
Crude protein (CP), g/kg DM	115	115	114	0.9	0.85
aNDFom, g/kg DM	180	179	170	5.7	0.45
Ash, g/kg DM	21	21	21	0.2	0.71
Crude fat, g/kg DM	29	30	30	0.4	0.32
Physical properties[3]					
Bulk density, g/L	384 ± 10	497 ± 12	607 ± 12		
Radia expansion, %	33 ± 2	20 ± 2	12 ± 2		
Hardness, N	107 ± 24	67 ± 14	141 ± 18		
Specific density, g/ml					
Dry pellets	0.66 ± 0.05	0.80 ± 0.01	1.04 ± 0.01		
Wet pellets[4]	0.88 ± 0.04	0.99 ± 0.04	1.22 ± 0.03		
Sinking velocity[5], mm/sec	-	27 ± 1 (60)	110 ± 3 (100)		
Fluid stability index (FSI), %					
30 min incubation	98 ± 1.2	99 ± 0.6	99 ± 0.8		
60 min incubation	97 ± 0.6	98 ± 1.4	99 ± 0.4		
120 min incubation	88 ± 0.6	86 ± 0.3	92 ± 0.3		

540 [1] LD = Low density; MD = Medium density; HD = High density

541 [2] Standard error of the mean (n=3)

542 [3] Average values with standard deviations

543 [4] Determined after soaking pellets in rumen fluid for 30 min at 39 °C.

544 [5] Numbers in the parenthesis represent percentages of sinking pellets measured up to 20 min after dropping.

Table 2.

Composition and chemical analysis of partial mixed ration (PMR) (g/kg DM unless otherwise stated)

Item	
Ingredients	
Soybean meal	119
Rapeseed cake, rolled	36
Sugar beet pulp, dried, rolled	119
Grass/clover- silage (first cut)[a]	716
Mineral mix, type 1, granulated[b]	9
Nutrients	
DM, g/kg	380
Crude protein	216
Starch	6
Crude Fat	28
NDF	309
Ash	87

[a] Chemical analysis (Eurofins A/S, Vejen, Denmark): DM, 357 g/kg; ash 88.4 g/kg DM; aNDFom, 333 g/kg DM; CP, 174 g/kg DM; in vitro digestible OM, 799 g/kg OM.

[b] Premix lactation (VM 2, Vitfoss, Gråsten, Denmark) containing (per kg): 160 g of Ca, 50 g of P, 65 g of Mg, 90 g of Na, 0.5 g of S, 600 kIU of vitamin A, 190 kIU of vitamin D,

4000 IU of vitamin E, 4000 mg of Mn, 1500 mg of Cu, 25 mg

of Co, 4500 mg of Zn, 225 mg of I, and 50 mg of Se.

553 **Table 3.**

554 Nutrient intake (kg/day)

	Experimental concentrate pellets[1]				
Item	LD	MD	HD	SEM[2]	P-Value
Dry matter intake					
PMR	14.9	14.5	14.8	0.3	0.68
Experimental concentrate	4.40[b]	4.45[a]	4.40[b]	0.01	0.01
Total	19.3	19.0	19.2	0.3	0.74
Starch	2.87	2.85	2.84	0.01	0.33
Crude protein	3.71	3.66	3.71	0.06	0.79
aNDFom	5.41	5.25	5.34	0.14	0.76
Ash	1.38	1.35	1.38	0.03	0.74
Organic matter	17.9	17.6	17.8	0.30	0.74

555 [1] LD = Low density; MD = Medium density; HD = High density

556 [2] Standard error of the mean (n=3)

Table 4.

Flow and apparent digestibility of starch along the gastrointestinal tract

	Experimental concentrate pellets[1]				
Item	LD	MD	HD	SEM[2]	P-Value
Flow, g/d					
Duodenal starch	442	488	659	104	0.45
Duodenal glucose	15.1	4.1	16.3	5.2	0.37
Ileal starch	64	90	121	12	0.15
Ileal glucose	8.1	7.1	7.8	1.6	0.90
Fecal starch	26	36	40	3.9	0.23
Fecal glucose	25	18	21	3.0	0.43
Digestibility					
Rumen digestibility, % of intake	85	83	77	3.5	0.43
Small intestine digestibility					
% of entering	86	80	80	5.4	0.68
% of intake	13	14	19	3.6	0.58
Hindgut digestibility					
% of entering	59	52	64	14	0.85
% of intake	1.3	1.9	2.8	0.5	0.32
Total tract digestibility, % of intake	99	99	99	0.1	0.23

[1] LD = Low density; MD = Medium density; HD = High density

[2] Standard error of the mean (n=3)

Table 5.

Apparent digestibility (%) of dry matter (DM), nutrient detergent fiber (aNDFom), and crude protein (CP) along the gastrointestinal tract

	Experimental concentrate pellets[1]				
Item	LD	MD	HD	SEM[2]	P-Value
DM					
Rumen digestibility, % of intake	26	27	22	3.4	0.65
Small intestine digestibility					
% of entering	56	56	60	1.3	0.28
% of intake	42	41	46	2.5	0.41
Hindgut digestibility					
% of entering	17	24	25	7.0	0.71
% of intake	5.5	7.8	7.8	2.5	0.77
Total tract digestibility, % of intake	73	75	76	1.6	0.50
aNDFom					
Rumen digestibility, % of intake	72	71	70	1.4	0.59
Small intestine digestibility					
% of entering	-23	-28	-31	12	0.90
% of intake	-8.8	-6.8	-8.8	3.6	0.91
Hindgut digestibility					
% of entering	17	28	32	15	0.79
% of intake	8	10	14	6.4	0.81
Total tract digestibility, % of intake	71	74	74	2.9	0.69
Crude Protein					
Rumen digestibility, % of intake	-34	-32	-43	3.5	0.24
Small intestine digestibility					
% of entering	73[b]	71[b]	76[a]	0.4	0.02
% of intake	98[ab]	94[b]	109[a]	2.4	0.08
Hindgut digestibility					
% of entering	-3.0	13.6	12.8	7.8	0.41
% of intake	-1.0	5.1	4.3	2.8	0.41
Total tract digestibility, % of intake	63	67	70	2.5	0.33

[1] LD = Low density; MD = Medium density; HD = High density

[2] Standard error of the mean (n=3)

Table 6.

Postprandial rumen pH and VFA patterns (until 8 h after morning feeding)

	Experimental concentrate pellets[1]				P-Values		
Item	LD	MD	HD	SEM[2]	Trt	Time	Time×Trt.
Dorsal:							
pH	5.80	5.84	5.78	0.10	0.90	<0.01	0.44
Total VFA, mM	147	151	155	7.3	0.58	0.07	0.57
Acetate, % of total	58	58	59	1.3	0.16	<0.01	0.30
Propionate, % of total	22	22	21	0.8	0.23	0.01	0.68
Butyrate, % of total	15	15	15	1.4	0.92	0.05	0.41
Isobutyrate, % of total	0.70	0.75	0.73	0.04	0.41	0.26	0.65
Valerate, % of total	2.19	2.37	2.25	0.16	0.50	0.01	0.07
Isovalerate, % of total	1.48	1.54	1.57	0.20	0.74	0.43	0.64
Caproate, % of total	0.50	0.53	0.56	0.10	0.24	0.03	0.22
Acetate:Propionate ratio	2.66[b]	2.63[b]	2.84[a]	0.13	0.06	<0.01	0.52
L-lactate, mM	3.15	1.69	1.70	0.62	0.19	0.23	0.58
Medial:							
pH	5.63	5.67	5.68	0.11	0.86	0.02	0.85
Total VFA, mM	165	155	162	7.8	0.50	0.56	0.98
Acetate, % of total	57[a]	58[ab]	59[b]	1.1	0.03	0.02	0.88
Propionate, % of total	22	22	21	0.9	0.16	0.04	0.79
Butyrate, % of total	15	15	15	1.4	0.85	0.36	0.95
Isobutyrate, % of total	0.71	0.76	0.72	0.03	0.35	0.69	0.85
Valerate, % of total	2.30	2.37	2.27	0.14	0.89	0.07	0.97
Isovalerate, % of total	1.54	1.53	1.55	0.22	0.99	0.53	0.82
Caproate, % of total	0.52	0.53	0.56	0.11	0.27	0.16	0.95
Acetate:Propionate ratio	2.58[b]	2.67[b]	2.84[a]	0.13	0.03	0.01	0.81
L-lactate, mM	0.31[b]	0.84[a]	0.27[b]	0.20	0.06	0.07	0.81
Ventral:							
pH	6.57	6.46	6.51	0.08	0.32	0.30	0.62
Total VFA, mM	116	121	121	4.6	0.54	0.45	0.71
Acetate, % of total	59	59	61	1.5	0.08	0.44	0.95
Propionate, % of total	22	22	20	0.8	0.16	0.04	0.90
Butyrate, % of total	14	14	14	1.6	0.92	0.59	0.96
Isobutyrate, % of total	0.88	0.84	0.86	0.02	0.63	0.28	0.28
Valerate, % of total	2.05	2.17	2.00	0.14	0.65	0.06	0.78
Isovalerate, % of total	1.58	1.54	1.60	0.19	0.91	0.38	0.60
Caproate, % of total	0.44	0.48	0.49	0.10	0.13	0.16	0.64
Acetate:Propionate ratio	2.77[b]	2.77[b]	2.98[a]	0.13	0.08	0.03	0.95
L-lactate, mM	0.08	0.94	0.24	0.25	0.12	0.07	0.10

[1] LD = Low density; MD = Medium density; HD = High density

[2] Standard error of the mean (n=3)

570 **Table 7**.

571 Diurnal pH and VFA pattern in ventral rumen

	Experimental concentrate pellets[1]				P-Values		
Item	LD	MD	HD	SEM[2]	Trt	Time	Time×Trt.
pH	6.49	6.39	6.44	0.07	0.23	<0.01	0.77
Total VFA, mM	127	127	128	5.6	0.95	<0.01	0.80
Acetate, % of total	61	60	62	0.9	0.42	<0.01	0.94
Propionate, % of total	20.9^{ab}	21.2^{a}	20.3^{b}	0.7	0.04	<0.01	0.64
Butyrate, % of total	13	13	13	1.1	0.85	<0.01	0.99
Isobutyrate, % of total	0.80	0.77	0.76	0.02	0.22	<0.01	0.43
Valerate, % of total	1.93	2.07	1.93	0.07	0.34	<0.01	0.78
Isovalerate, % of total	1.49	1.48	1.46	0.16	0.52	<0.01	0.52
Caproate, % of total	0.41	0.45	0.44	0.08	0.16	<0.01	0.93
Acetate:Propionate ratio	2.97^{ab}	2.87^{b}	3.05^{a}	0.10	0.09	<0.01	0.96
L-lactate, mM	0.87	0.87	1.34	0.35	0.48	<0.01	0.82

572 [1] LD = Low density; MD = Medium density; HD = High density

573 [2] Standard error of the mean (n=3)

Table 8.

Postprandial duodenal dry matter and starch flow

	Experimental concentrate pellets[1]				P values		
Item	LD	MD	HD	SEM[2]	Trt	Time	Time*Trt.
Intake[3]:							
Dry matter, kg/d	17.2	15.6	18.2	0.50	0.13		
Starch, g/d	2.87	2.85	2.84	0.01	0.33		
Digesta flow up to 9h after feeding:							
Dry matter, g/h	549[a]	477[b]	498[b]	111	0.01	0.28	0.74
Starch, g/h	13.6	11.8	14.9	3.94	0.53	0.02	0.36
Starch, g/kg DM	25.5	24.2	29.6	4.30	0.14	<0.01	0.16
Digesta flow up to 16h after feeding:							
Dry matter, g/h	546[a]	488[b]	522[a]	105	0.01	0.11	0.71
Starch, g/h	14.4[b]	14.0[b]	18.3[a]	4.21	0.05	<0.01	0.56
Starch, g/kg DM	25.8[b]	28.1[b]	34.0[a]	4.3	0.01	<0.01	0.39

[1] LD = Low density; MD = Medium density; HD = High density

[2] Standard error of the mean (n=3)

[3] Intake on the day for postprandial sampling

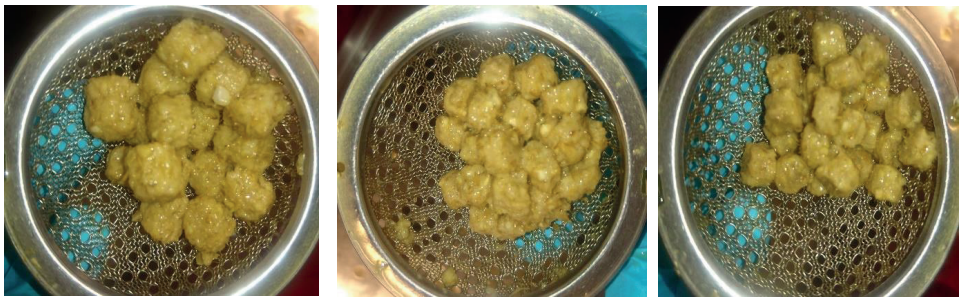
Table 9.

Daily gas exchange

	Experimental concentrate pellets[1]				
Item	LD	MD	HD	SEM[2]	P-Value
CH_4, L/d	595	535	536	13.9	0.14
CO_2, L/d	7070	6844	6612	114	0.20
H_2, L/d	5.12	4.62	4.56	0.53	0.74
CH_4, L/kg DMI	31.8	30.5	32.4	1.70	0.74

[1] LD = Low density; MD = Medium density; HD = High density

[2] Standard error of the mean (n=3)

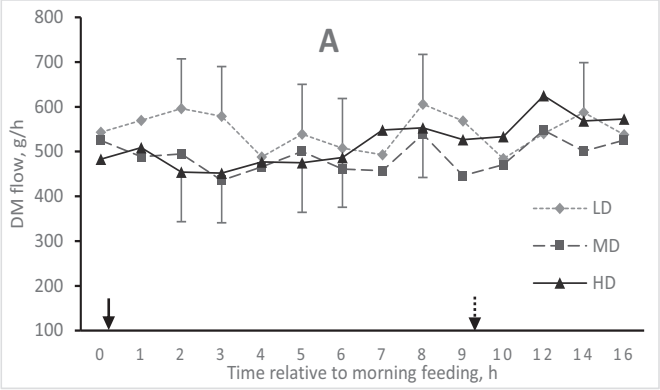


584 (LD) (MD) (HD)

585 **Figure 1.** Example of fluid stability of extruded pellets with low-density (LD), medium-density (MD), and high-
586 density (HD) after 120 min of incubation in rumen fluid.

587

588



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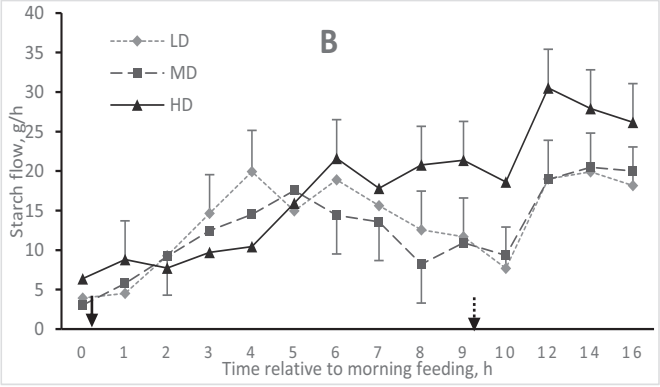

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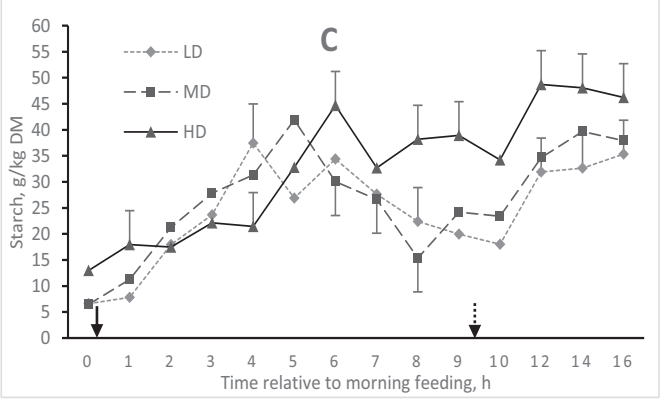

591 **Figure. 2**. Postprandial duodenal flow of dry mater (A) and starch (B) and starch concentration (C) for extruded
592 pellets with low-density (LD), medium-density (MD), and high-density (HD). Solid arrow indicates morning
593 feeding and dashed arrow indicates afternoon feeding of experimental concentrate pellets.

Paper-III

Effects of density and fluid stability of extruded barley-soybean meal pellets on digestion kinetics and rumen fermentation pattern in dairy cows

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Abstract

The effects of physical functional properties of feed pellets on nutrient digestion kinetics were investigated by measuring digestibility, postprandial duodenal appearance of starch and protein, and postprandial rumen fermentation patterns in dairy cows fed a basal diet low in starch. Four treatment concentrate pellets were produced based on a compound concentrate meal containing 70% barley and 30% soybean meal (SBM; as-is basis). One treatment was pelleted by conventional pelleting after expander processing and expressed as high-density conventional (HDcon) pellets, whereas the other treatments were extruded using three distinct settings giving pellets with either high-density (HDext), medium-density (MDext), or low-density (LDext). Conventional pellets had a markedly lower fluid stability index (FSI) compared with extruded pellets. The animal experiment was conducted in a 4×4 Latin square design using four lactating Norwegian Red cows fitted with ruminal and duodenal cannulas. Two cows were also fitted with an ileal cannula. Over three days, eight samples from duodenal and ileal digesta and total feces were collected to determine digestibility. For postprandial duodenal starch and protein appearance, fifteen sample sets of duodenal chyme were obtained from each cow at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 17 h, whereas for postprandial rumen fermentation patterns, nine sample sets of rumen dorsal, medial and ventral fluid were taken from each cow at 0, 1, 2, 3, 4, 5, 6, 7, 8 h relative to morning feeding of the experimental concentrate at 07:00. Rates of digestion (k_d) and passage (k_p) of starch were estimated using total rumen evacuation. Rumen degradation of starch and protein was also determined by *in situ* technique. The RSD was lower for high-density pellets than other density pellets (87% *versus* 90%), but it did not differ between HDcon and HDext despite marked differences in FSI. Similarly, the postprandial duodenal appearance of starch was highest for high-density pellets, with a more rapid appearance for HDcon than for HDext. Nevertheless, k_p of starch determined by rumen evacuation did not differ

among treatments. Diurnal and postprandial dorsal and medial rumen pH patterns reached a lower nadir for extruded pellets than conventional pellets. Total VFA concentration in rumen did not differ among treatments, but propionate concentration was highest for LDext in the dorsal rumen. The total tract digestibility of starch was more than 99% for all treatments, indicating a high intestinal digestibility of starch with all pellet types. In contrast to rumen starch kinetics, where no difference between treatments was found, the k_d of protein was the lowest for LDext. The duodenal protein flow was higher for extruded pellets, being highest for LDext, than conventional pellets. Ruminal digestibility of neutral detergent fiber (NDF) was lower for extruded pellets than conventional pellets, but the total tract digestibility of NDF did not differ among treatments. In conclusion, the present study indicated that the physical properties of feed pellets could alter the site of nutrient digestion in the gastrointestinal tract of dairy cows, where the density of pellets was the most crucial property governing the escape from the rumen to the small intestine, while FSI of pellets had limited effects. Indeed, further investigations are needed.

Abbreviations: Ac:Pc, acetate:propionate ratio; CP, crude protein; DM, dry matter; DMI, dry matter intake; ECM, energy corrected milk; EPD, effective protein degradability; ESD, effective starch degradability; FSI, fluid stability index; HDcon, high density conventional; HDext, high-density extruded; k_d , rate of degradation; k_p , rate of passage; LDext, low-density extruded; MDext, medium-density extruded; MCP, microbial crude protein; aNDFom, ash-free neutral detergent fiber; *Pd*, potentially degradable fraction; RPM, rotations per min; RSD, ruminal starch digestibility; *S*, soluble fraction; SBM, soybean meal; ISD, intestinal starch digestion; TTSD, total tract starch digestion; VFA, volatile fatty acids;

Keywords: In vivo; Processing; Rumen degradation; Rumen passage; Starch, Protein

1. Introduction

To meet the nutrient demand for milk synthesis, high producing dairy cows are fed increasing quantities of concentrates. Starch is the predominant nutrient in concentrates, providing energy for the cow and microbial protein synthesis. However, high levels of rapidly fermentable starch may lead to rumen acidosis and health problems resulting in decreased fiber digestibility, microbial protein synthesis, and production (Krause and Oetzel, 2006). Shifting the starch digestion site from the rumen to the small intestine could improve the rumen environment and microbial efficiency. In addition, the energetic efficiency of starch is higher when digested in the small intestine compared to the rumen (Owens et al., 1986; Huntington et al., 2006; Reynolds, 2006). Moreover, the protein value of feedstuffs can be improved by increasing both microbial protein synthesis and rumen escape of dietary protein (Tamminga et al., 2007). Thus, partially shifting the site of digestion of starch and protein digestion from the rumen to the small intestine may improve feed utilization and production in dairy cows.

The site of nutrient digestion is commonly shifted by altering the rumen digestion rate, either by selecting feed ingredients or applying various feed processing techniques. However, as the extent of rumen digestion results from the concurrent fractional rate of digestion and fractional rate of passage, also manipulating the fractional rate of passage could potentially be a key method to alter the digestion site. Compared to the rate of digestion, altering the rate of passage is scarcely studied. It has been demonstrated by the use of inert plastic particles or labeled indigestible plant fiber particles that the rate of passage of rumen particulate matter is mainly dependent upon particle density. In this regard, high-density (sinking) particles have a higher probability of passage from reticulorumen than low-density (floating) particles (Campling and Freer, 1962; desBordes and Welch, 1984; Kaske and Engelhardt, 1990; Dufreneix et al., 2019).

A slowly degradable floating particle may have less probability of rumen escape but may improve synchronization between nutrient release and demand. Likewise, feed pellets with a specific density designed to increase passage rate may alter the site of nutrient digestion and benefit the rumen environment. Recently, Larsen et al. (2019) suggested that feed pellets with high density and high fluid stability could increase postprandial duodenal nutrient flow, thereby influencing rumen digestion kinetics.

The present study aimed to investigate the effects of physical properties (i.e., density and fluid stability) of concentrate pellets on the rumen environment as well as digestion and passage kinetics of starch, protein, and fibers in dairy cows. We hypothesized that compared to conventional pellets, extruded pellets 1) with high density and high fluid stability will increase the rate of passage, resulting in increased rumen escape of starch and protein, whereas 2) pellets with low density and floating nature will have less probability of escape, but may improve rumen environment through greater starch fermentation in dorsal and medial rumen compartments and thereby reducing local acidic conditions in the ventral rumen as assessed by rumen fermentation variables.

2. Materials and Methods

The animal experiment was performed at the metabolism unit at the Norwegian University of Life Sciences (NMBU) in Ås, Norway, following laws and regulations controlling experiments on live animals and under the surveillance of the Norwegian Animal Research Authority (FOTS ID. 12399, ref. 17/85015). Processing of experimental concentrate was carried out at Center for Feed Technology (FôrTek) at NMBU under the approval of the Norwegian Food Safety Authority (Mattilsynet Case ID: 2017/252266-3).

93 *2.1. Animals and experimental design*

94 Four multiparous Norwegian Red cows in early lactation (64±10 days post-partum), weighing
95 611 ± 43 kg and with an average milk yield of 34 ± 6 kg day^{-1} at the start of the experiment were
96 used in a 4 x 4 Latin square design, balanced for carry-over effects, with four treatments, cows,
97 and periods. Each period consisted of 21 days, of which the first 11 days were used for adaption
98 and the last 10 days were used for sampling. All cows were fitted with a rumen cannula (Bar
99 Diamond Inc., Parma, Idaho, USA; inner diameter: 100 mm) and an open T-piece duodenal
100 cannula (made of PVC with an inner diameter of 25 mm) in the proximal duodenum, 50-60 cm
101 distal to the pylorus. Two cows were also fitted with an open T-piece ileal cannula located ca.
102 20 cm cranial to the caecum. Cows were housed in tie stalls with rubber mats and had *ab libitum*
103 access to fresh water from individual water bowls.

104 *2.2. Processing and technical analysis of experimental concentrate*

105 The experimental concentrate used in four treatments consisted of 70% barley grain and 30%
106 solvent-extracted soybean meal (SBM, obtained from Denofa AS, Fredrikstad, Norway). Barley
107 and SBM were ground by a hammer mill (E-22115 TF, Muench-Wuppertal, Germany) to pass
108 a 2 mm screen and subsequently mixed with a twin shaft paddle mixer (Forberg AS, Larvik,
109 Norway) for 300s. Thereafter, the blend was divided into four portions giving the four
110 treatments. One portion was processed using an annular gap expander (Kahl OE 23, Reinbek,
111 Germany) at 110 °C and 10 bar prior to being pelleted with conventional pellet press (Pellet
112 Press, RPM 350.100, Munch-Edelsthal, Wuppertal, Germany) using 5 mm die size. This
113 treatment constituted the control and, since the pellets have high density, is expressed as the
114 high-density conventional (HDcon) treatment. The remaining portions were pre-conditioned in
115 a double shaft conditioner (BCTC 10, Bühler, Uzwil, Switzerland) and extruded (Twin Screw

BCTG 62 Extruder, Bühler, Uzwil, Switzerland; 5 sections) using 3 mm die size (revolver die; 12 number of dies). Three distinct processing settings were used to obtain three extruded treatments with either low-, medium- or high-density pellets. The low-density extruded (LDext) treatment was produced using high screw speed (275 RPM) and injecting steam at section 4 of the extruder barrel, giving an exit temperature of 125 °C and high extrudate expansion. The medium-density extruded (MDext) treatment was obtained by reducing the screw speed (210 RPM), giving exit temperature of 111 °C, and medium extrudate expansion. The high-density extruded (HDext) treatment was produced by using low screw speed (210 RPM) and cooling at the last section (section 5) of the extruder barrel, giving an exit temperature of 88 °C and low extrudate expansion. Maximum temperatures and die pressures obtained during extrusion processing were 128, 113, 109 °C and 25, 40, 41 bar for LDext, MDext, and HDext treatments, respectively. The extruded treatments were dried in a fluid bed continuous dryer (FôrTek, NMBU) at ~100 °C for 6-8 min and subsequently cooled using mobile batch coolers (FôrTek, NMBU).

During steady-state processing, samples were obtained for each treatment at the start, middle, and end of the production. These three samples were pooled into one sample for each treatment to analyze physical properties. The physical properties analyzed were bulk density, expansion, hardness, specific density, sinking velocity, and fluid stability index (FSI) (Table 1). Bulk densities were determined in triplicate by measuring pellets' weight in a 1 L steel cylinder, as described by Sørensen (2012). Radial expansion of pellets was calculated as the ratio between average diameter of a pellet, measured at three points with an electronic Vernier caliper, and diameter of the die. Each reported value is the average of 30 measurements. Hardness was determined with a texture analyzer HK5T (Tinius Olsen Ltd., UK) fitted with a 100 N load cell

using either a flat knob or a knife knob at a compression speed of 10 mm/min. The force (N) measured at the first crack in a pellet was used as hardness value and is the average of 15 measurements. The specific density, sinking velocity, and FSI were analyzed as described by Khan et al. (2021a).

2.3. Animal experiment

2.3.1. Feeding of animals and feed sampling

Feeding of animals was comprised of grass silage and concentrate. In addition, a multimineral mix (Pluss Storfe multitilskudd, Felleskjøpet, Agri, Lillestrøm, Norway) was spread over the silage at each feeding to yield 200 g per day and animal. The silage used was a mixture of two types blended in a TMR-mixer wagon (Kverneland Duo 1814, Klepp, Norway) at a ratio of 50:50 on wet basis (Table 2). Silage 1 was a second cut with dry matter (DM) content of 37.6% and crude protein (CP) and neutral detergent fiber (NDF) contents of 112 and 510 g/kg DM, respectively, whereas silage 2 was the first cut with DM of 19.9% and CP and NDF contents of 158 and 533 g/kg DM, respectively. The silage mixture was fed *ad libitum* (10% refusals) and was offered at 7:30, 15:30, and 21:00 h at a ratio of 0.4, 0.4, and 0.2 of expected daily intake, respectively. For concentrates, a fixed daily ration of 10 kg (as is basis) per cow was used. The ration consisted of 7 kg of one of the four experimental treatments and 3 kg of a commercial compound concentrate (FORMEL Favør 80, Felleskjøpet Agri, Lillestrøm, Norway; Table 2). The daily concentrate ration was divided into three equal meals, each consisting of 1.0 kg of the commercial compound and 2.3 kg of experimental treatment. The rations were offered at 7:00, 15:00, and 20:30 h in each period, except day 16 when 10 kg of 100% experimental concentrate divided into two equal meals was fed at 07:00 and 15:00 h.

In each period, one sample of each concentrate and ten samples of the offered and refused silage mixture were taken during weeks 2 and 3 and subsampled for DM determination and chemical analysis (stored frozen at -20 °C). DM was determined by drying for 24 hours at 103 °C to estimate DM intake (DMI). At the end of each period, silage samples for chemical analysis were pooled and mixed within the period and stored frozen at -20 °C until freeze-drying. At the end of the experiment, concentrate samples for each treatment from periods 1 and 2 were pooled together, whereas samples from periods 3 and 4 were pooled together before drying. Feed (silage and concentrate) samples were ground and analyzed for DM, starch, nitrogen (N), NDF, ash, and fat.

2.3.2. Digestibility and post-prandial digesta flow

To measure the intestinal flow of digesta, a dual-marker technique was applied using chromium ethylenediaminetetraacetic acid (Cr-EDTA) and ytterbium acetate (Yb-acetate) as external markers for the liquid and particulate phase, respectively (Faichney, 1975). Solutions of Cr-EDTA (3.0 kg) and Yb-acetate (3.0 kg) markers were pulse dosed at ventral sac in the rumen at 09:00 on day 4, whereupon markers were continuously infused at the rate of ca. 3 kg d^{-1} until 23:45 on day 16 in each period, using a peristaltic pump. The concentrations of Cr and Yb in the solutions were 906 ± 24 mg kg^{-1} and 845 ± 46 mg kg^{-1}, respectively, giving a daily infusion of 2.72 ± 0.02 g Cr and 2.52 ± 0.05 g Yb.

For rumen digestibility determination, 8 samples of duodenal digesta (500 mL) were collected on day 13 (09:00, 15:00, and 22:00), day 14 (04:00, 12:00, and 18:00), and day 15 (01:00 and 13:00), using tube-shaped plastic bags mounted to the cannula with a plastic knee. Corresponding to duodenal digesta sampling, 300 mL of ileal digesta was also collected from the two cows. After pH measurement, samples were pooled within cow and period and stored

frozen at -20 ºC until freeze-drying. Finally, the freeze-dried samples were ground, and digesta was analyzed for DM, ash, starch, N, NDF, Yb, and Cr.

From 08:00 on day 12 until 08:00 on day 15 (72 hours), feces and urine were quantitatively collected to determine the total tract digestibility and N utilization. Urine was separated from feces in buckets at spontaneous excretion. Urine and feces accidentally not collected this way were monitored. All materials were immediately transferred to collection buckets, which were changed every 8 hours and kept at 4 ℃. The urine was acidified with 0.5 L 10% sulphuric acid to keep pH below 4. Every 24 hours, urine was manually mixed within the cow, and 10% was transferred to a container and kept frozen at -20 ℃. Feces was mixed within the cow using a concrete blender for 3 min, after which 10% was transferred to a container and kept frozen at -20 ℃. After each period, feces and urine samples were thawed and subsequently mixed within cow and period before samples were taken and kept frozen at -20 ℃. After freeze-drying, fecal samples were ground and analyzed for DM, ash, starch, N, NDF, Yb, and Cr. Urine was analyzed for N and Cr.

For postprandial digesta flow on day 16, cows were fed 100% experimental concentrate as described above. Cows were allowed to eat experimental concentrate for one hour. Two cows were eating all concentrate within 15 min. The two other cows were eating more slowly, but except for one cow not eating HDext, concentrates were consumed nearly all within 1 hour. The cow not eating HDext was considered missing in statistical analysis. Samples of duodenal chyme were obtained from each cow at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 17 h relative to the feeding of concentrate at 07:00. Samples (about 300 mL in each) were immediately transferred to pre-weighed aluminum trays. About 10 mL of stirred digesta was transferred to a 15 mL polystyrene tube and stored at −20 °C for Cr determination. The remaining sample was

weighed after pH determination. The trays with digesta were kept frozen at −20 °C until freeze-drying and subsequently weighing and grinding before analyzing for DM, starch, and N.

2.3.3. Ruminal measurements

On day 18, rumen liquid for the determination of postprandial volatile fatty acids (VFA), pH and ammonia, was withdrawn by suction strainer (#RT, Bar Diamond Inc., Parma, Idaho, USA) from the ventral, medial, and dorsal sac of the rumen. The samples were taken at each hour from 07:00 (before feeding) until 15:00 constituting a total of 9 samples from each position in the rumen and 27 samples per cow per period in total. Sampling was conducted by withdrawing about 40 mL of rumen fluid from the ventral sac using the suction strainer and a 50 mL syringe. The sample was transferred to a 50 mL Falcon tube. Following the same procedure, the strainer was then pulled upward about 25-30 cm to get a sample from the medial rumen, whereas the sample from the dorsal rumen was taken with a suction strainer through or just below the upper fiber mat. The pH in rumen fluid samples was measured immediately by a pH meter (WTW 3320, Weilheim, Germany) fitted with an electrode and calibrated at pH 4.000 and 7.000. Thereafter, 9.5 mL of rumen fluid was transferred to a 15 mL polystyrene tube containing 0.5 mL of formic acid (analytical grade) as a preservative. The tubes were closed and turned upside down once for blending before storing at 4 °C until analysis.

To determine the diurnal rumen pH variations, pH was logged every 10^{th} min for 24 hours starting from 15:00 on day 18 with pH-meters (WTW 3320, Weilheim, Germany) equipped with electrodes attached to a stainless-steel sink. The electrodes were placed in a perforated tube fitted to the cannula lid, locating the electrodes 10-15 cm above the bottom of the ventral rumen sac.

For measurement of rumen liquid passage rate, a pulse dose of Cr–EDTA was administered at 07:00 h on day 18 in each period. Representative samples (10 mL in each) of rumen liquid were taken at 0 (before administration), 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 14, 17, 23, 29 and 37 h after administration of the pulse dose. The samples were kept frozen at −20 °C and later analyzed for Cr.

Rumen contents of liquid and particulate matter were determined by manual evacuation through the rumen fistula at 09:00 h on day 20 and at 13:00 h on day 21. The rumen evacuation was performed as described by Prestløkken and Harstad (2001). At each evacuation time, four samples were composited on a weight basis from subsamples of rumen liquid and mat. Two samples were dried at 103 °C for 24 hours to determine DM. The other two were immediately frozen (−20 °C) and freeze-dried before grinding and analysis of starch.

2.3.4. In situ experiment

The *in situ* experiment was conducted from day 5 to day 7 in period 4. Rumen degradation of starch and protein for all four experimental treatments was determined in each cow. The procedure was as described in the NorFor system (Åkerlind et al., 2011) except that pellets were not ground and not all incubation times were used. The bags (SEFAR Nitex 03-37/24, Heiden, Switzerland), containing the 2 g of pellets from each treatment, were incubated in the rumen for 0 h (4 bags of each treatment only washed in the washing machine), 4 h (2 bags), 8 h (3 bags), 24 h (5 bags) and 48 h (6 bags). After incubation and drying, pooled residues within treatment, cow, and time were milled for 30 seconds at a frequency of 50 Hz using a Retsch Mixer mill (Retsch, Haan, Germany) and stored in air-tight glass jars at room temperature until analysis of starch and N.

2.3.5. Milk recording and sampling

The cows were milked at 06.30 and 19.30, and yield was recorded using the Tru-Test Milk Meter (Datamars SA, Lamone, Switzerland). In each period, separate aliquot samples were taken during a.m. and p.m. milking at days 11, 12, 18, and 19 and transferred to 40 mL "Ola-beger", prepared with Bronopol (2-bromo-2-nitro-1,3-propanediol, Broad Spectrum Microtabs® II). Samples were kept cold at 4 °C until analyzed for milk protein, fat, lactose, urea, and free fatty acid (FFA) contents and somatic cell count (SSC) by Fourier Transform Infrared (FTIR) spectroscopy using MilkoScan™ Combifoss 6500 instrument (Foss, Hillerød, Denmark) at TINE laboratory (Brumunddal, Norway).

2.4. Chemical analysis of samples

For chemical analysis, all silage and digesta samples were freeze-dried except for concentrate and *in situ* residue samples, which were dried at 45 °C for 48 hours. Except for starch which was ground on a 0.5 mm screen, dried samples for analysis of nutrients were ground on a 1 mm screen using a Retsch SM 200 cutting mill (Retsch GmbH, Haan, Germany). Ash was analyzed using the ISO 5984 method (550 °C for a minimum of 4 h). Crude fat was analyzed by accelerated solvent extraction (ASE350, Dionex Corporation, Sunnyvale, CA, USA) as described in European Commission Regulation EC No. 152/2009 (EC, 2009). Starch was analyzed enzymatically using AACCI (1999) Method 76–13.01 (Megazyme amyloglucosidase/α-amylase method), and liberated glucose was determined spectrophotometrically by Maxmat PLII (Maxmat SA, Montpellier, France) using enzymatic endpoint reaction (Hexokinase) forming NADH. Starch content was corrected for free glucose by washing the original sample with 80% ethanol before enzymatic hydrolysis. Nitrogen was analyzed as Kjeldahl-N using Method 2001.11 (AOAC, 2002) according to (Thiex et al., 2002)

with Kjeltec 2400/2460 Auto Sampler System (Foss Analytical, Hillerød, Denmark). The NDF was determined with an ANKOM220 fiber analyzer (ANKOM Technology, Fairport, NY, USA) according to Mertens (2002) using sodium sulfite and heat-stable α-amylase. The samples were corrected for residual ash, and the results are presented as aNDFom. Analysis of chromium and ytterbium were carried out by the MP-AES method (Agilent 4200 MP-AES, Agilent Technology, Melbourne, Australia). Rumen fluid VFA were analyzed by gas chromatography (TRACE 1300 Gas Chromatograph equipped with Stabilwax-DA column 30 m, 0.25 mm i.d., 0.25 µm; Thermo Fischer Scientific S.p.A., Milan, Italy). Ammonia nitrogen (NH_3-N) in the rumen fluid was analyzed using Method 2001.11 (AOAC, 2002) according to Thiex et al. (2002) with a modification that block digestion was not carried out.

2.5. Calculations

The CP was estimated as N x 6.25. The daily DM flow of duodenal and ileal digesta was calculated from the daily infusion of marker (mg/d) over marker concentration in digesta (mg/kg DM) as the average flow estimates for the two markers. Based on DM flow, apparent digestibility of nutrients up to the duodenum (ruminal digestibility) and up to the ileum (ileal digestibility) was calculated from the intake and flow of nutrients at each site. The apparent intestinal digestibility was calculated based on inflow (at duodenum) and outflow (in feces) of nutrients. The apparent total tract digestibility was estimated from intake and collected fecal output. The postprandial duodenal DM flow was calculated assuming constant hourly rumen outflow of Cr. Subsequently, the postprandial duodenal flow of starch and CP was calculated from the contents of starch and CP present in DM at each time point. 17 h bypass starch from postprandial samples was estimated by multiplying each flow measurement with 17 and subsequently divided by starch intake, not corrected for basal starch flow.

297 Time below the certain pH limits was calculated by summing pH registrations below these limits
298 over a period of 1440 min (24 h) using 10 min intervals and converting the time back to h/d.

299 The rumen passage rate of liquid was estimated based on rumen disappearance of Cr after
300 administration of a pulse dose assuming an exponential dilution (Faichney, 1975) using linear
301 regression analysis of natural logarithm transformed Cr concentrations on data from 1 to 37 h
302 post-administration of the pulse dose.

303 The rumen evacuations were used to calculate the average rumen pool size of DM and starch.
304 Together with daily intake (kg d^{-1}) and duodenal flow data (kg d^{-1}), rumen pool size (kg) was
305 used to calculate fractional ingestion rate (k_i, h^{-1}), passage rate (k_p, h^{-1}) and degradation rate (k_d,
306 h^{-1}) using the equations: k_i= (daily intake/rumen pool size)/24, k_p= (daily flow to
307 duodenum/rumen pool size)/24 and $k_d = k_i - k_p$, respectively (Stensig et al., 1998).

308 *In situ* rumen degradation of starch and CP was estimated using the NLIN procedure of SAS
309 (SAS, 2013). The *in situ* data were fitted to the exponential equation $Y_t = S + Pd(1 - e^{-kdt})$, where
310 Y_t is degraded portion after t hours, S is the material water-soluble and immediately degraded
311 (%), Pd is the material potentially degradable over time (%), k_d (h^{-1}) is the fractional degradation
312 rate of Pd and t is incubation time (h). the effective degradability of starch and protein in the
313 rumen were estimated as described by Ørskov and McDonald (1979) using the equation: $ED\ (\%) = 100 \times [S + (Pd \times k_d)/(k_d + k_p)]$, where S, Pd, and k_d are described above, and k_p is the
315 fractional rate of passage assumed to be either 0.08 or 0.05 h^{-1} (Madsen et al., 1995). *In situ*
316 rumen escape of starch and protein was calculated as the difference between daily intake and
317 rumen degradation estimate. The effective starch degradability (ESD) and the effective protein
318 degradability (EPD) for commercial compound concentrate were 88% and 81% for k_p of 0.05 h^{-1} and 84% and 69% for k_p of 0.08 h^{-1}, respectively. EPD for grass silage was assumed to be 85%

according to feed tables (Luke, 2015). Microbial protein flow was estimated from the difference of *in situ* rumen escape of dietary protein and the daily flow of protein into the duodenum. Microbial protein flow was corrected for endogenous protein flow according to Volden and Larsen (2011) using 30 g endogenous CP per kg organic matter flow.

Energy-corrected milk (ECM) yield was calculated for individual cows based on observed milk yield and the analyzed contents of fat, protein, and lactose (Sjaunja et al., 1991).

2.6. Statistical analysis

Feed intake, nutrient digestibility, and rumen evacuation data were statistically analyzed with the MIXED procedure of SAS (2013) using a model with period and treatment (Trt) as fixed effects and cow as a random effect. The postprandial duodenal digesta flow, rumen fermentation variables, and milk data were analyzed using the MIXED procedure of SAS for repeated measurements using a model with the period, Trt, Time relative to feeding (or Day in case of milk data), and Trt × Time (or Trt × Day) as fixed effects, and cow as a random effect. Kenward-Roger method was used to calculate denominator degrees, and Time within cow × period was considered a repeated measurement using the spatial power covariance structure chosen over autoregressive order 1 (AR1), heterogenous autoregressive order 1 (ARH1), and antedependence order 1 (ANTE1) based on Akaike information criteria (AIC). The duodenal starch concentration and flow were square-root transformed, whereas milk somatic cell count was $\log_{10}$ transformed to obtain a normal distribution of residuals. The *in situ* data was analyzed with the GLM procedure of SAS using a model with treatment and cow as fixed effects. The following predefined contrasts were tested: High density (HDcon and HDext) pellets versus other density (LDext and MDext) pellets, expressed as HD × LMD and conventional (HDcon) pellets versus extruded (LDext, MDext, and HDext) pellets, expressed as Con × Ext. The results

are reported as least square (LS) means with standard error of the mean (SEM). Treatment effects were judged using the PDIFF statement, and significance was claimed when $P \leq 0.05$, whereas tendencies were considered at $0.05 < P \leq 0.10$.

3. Results

3.1. Chemical composition and physical properties of experimental concentrates

Size (length x diameter; mm) of pellets were 5.9 x 5.2, 5.6 x 4.7, 5.5 x 4.1 and 10.0 x 5.0 for the LDext, MDext, HDext and HDcon treatments, respectively. The extruded pellets varied in their bulk densities and specific densities from 410 to 650 g/L and 0.66 to 1.07 g/mL, respectively (Table 1), giving pellets with floating (LDext), slow sinking (MDext), and fast sinking (HDext) properties. The conventional pellets (HDcon) showed high density (670 g/L) and fast sinking behavior but low fluid stability compared to extruded pellets (Figure 1). In HDcon, more than 50% of pellets were disintegrated after 30 min, whereas all extruded pellets showed higher than 85% FSI after 60 min incubation.

3.2. Feed intake

Concentrate and silage intake did not differ among treatments ($P_{Trt} \geq 0.13$; Table 3). Likewise, total DMI was not affected by treatments ($P_{Trt} = 0.82$). The intake of other nutrients did not differ among treatments ($P_{Trt} \geq 0.38$), except fat that was higher ($P_{Trt} = 0.05$) for HDcon compared to MDext and LDext treatments.

3.3. Digestibility of the main nutrients

3.3.1. Starch

363 The duodenal flow of starch tended to be higher for HDext than MDext and LDext treatments
364 (P_{Trt} = 0.09; Table 4) and was higher for high-density pellets compared with other density pellets
365 ($P_{HD \times LMD}$ = 0.02). Ruminal starch digestibility (RSD) differed among treatments (P_{Trt} = 0.02),
366 being lower for the HDext compared to MDext and LDext treatments and for high-density
367 pellets compared with other density pellets ($P_{HD \times LMD}$ = 0.01). However, the RSD of extruded
368 pellets did not differ from the conventional pellets ($P_{Con \times Ext}$ = 0.29). Intestinal starch
369 digestibility (ISD) was higher for HDext compared with MDext and LDext treatments ($P_{Trt} \leq$
370 0.07) and for high-density pellets compared with other density pellets ($P_{HD \times LMD} \leq 0.03$). More
371 than 98% of ingested starch was digested up to distal ileum with no difference among treatments
372 (Table 4). The total tract starch digestibility of starch (TTSD) was more than 99% for all
373 treatments, being higher for high-density pellets than other density pellets ($P_{HD \times LMD}$ = 0.03).

374 *3.3.2. Protein*

375 The duodenal flow of CP did not differ among treatments (P_{Trt} = 0.15; Table 4) but tended to be
376 lower for high-density pellets than for other density pellets ($P_{HD \times LMD}$ = 0.08) and for
377 conventional pellets compared with extruded pellets ($P_{Con \times Ext}$ = 0.10). Ruminal digestibility of
378 CP was negative, differing among treatments ($P_{Trt} < 0.01$) being lowest for LDext treatment and
379 was lower for extruded pellets as compared with conventional pellets ($P_{Con \times Ext} < 0.01$).
380 Intestinal digestibility of CP in % of entering did not differ among the treatments (P_{Trt} = 0.12)
381 but was higher for extruded pellets than conventional pellets ($P_{Con \times Ext}$ = 0.04). In % of intake,
382 intestinal digestibility of CP differed among treatments ($P_{Trt} < 0.01$) and was highest for LDext
383 and MDext pellets followed by HDext, making it higher for extruded pellets compared with
384 conventional pellets ($P_{Con \times Ext} < 0.01$). The Ileal and total tract digestibility of CP did not differ
385 among treatments ($P_{Trt} \geq 0.62$).

386 *3.3.3. Dry matter, organic matter, and NDF.*

387 The duodenal flow of DM and organic matter (OM) tended to be higher for extruded pellets than
388 conventional pellets ($P_{Con \times Ext} \leq 0.09$; Table 5). Ruminal digestibility of DM was higher for
389 HDcon than LDext, MDext, and HDext treatments ($P_{Trt} < 0.01$). Similarly, OM ruminal
390 digestibility was affected by treatments ($P_{Trt} = 0.02$) and was higher for conventional pellets
391 than for extruded pellets ($P_{Con \times Ext} = 0.01$). Intestinal digestibility of DM and OM differed among
392 treatments ($P_{Trt} \leq 0.06$) and was higher for extruded pellets than for conventional pellets ($P_{Con \times}$
393 $_{Ext} \leq 0.01$). The total tract digestibility of DM was higher ($P_{Trt} = 0.05$), whereas total tract OM
394 digestibility tended to be higher ($P_{Trt} = 0.06$) for MDext than other treatments.

395 Ruminal digestibility of NDF did not differ among treatments ($P_{Trt} = 0.11$; Table 5) but tended
396 to be higher with conventional pellets than extruded pellets ($P_{Con \times Ext} = 0.07$). No difference in
397 total tract digestibility of NDF was observed among treatments ($P_{Trt} = 0.43$), but post rumen
398 digestion of NDF tended to be higher with LDext than with HDcon treatment ($P_{Trt} = 0.06$) and
399 was higher with extruded pellets than conventional pellets ($P_{Con \times Ext} = 0.03$).

400 *3.4. Ruminal measurements*

401 Postprandial pH in dorsal rumen differed among treatments ($P_{Trt} = 0.01$; Table 6) and was higher
402 for high-density pellets than for other density pellets ($P_{HD \times LMD} < 0.01$). In the medial and
403 ventral rumen, pH did not differ among treatments ($P_{Trt} \geq 0.15$), but in the dorsal and medial
404 rumen, pH was higher for conventional pellets than for extruded pellets ($P_{Con \times Ext} \leq 0.04$). The
405 concentration of propionate in the dorsal rumen was higher for LDext treatment than for other
406 treatments ($P_{Trt} = 0.05$). The butyrate concentration tended to be higher for HDcon than LDext
407 treatment in the dorsal and ventral rumen ($P_{Trt} \leq 0.09$). It was higher for conventional pellets

than for extruded pellets ($P_{Con \times Ext} \leq 0.02$). The iso-butyrate concentration was lower for LDext than other treatments at all positions in the rumen ($P_{Trt} \leq 0.04$). The postprandial acetate:propionate (Ac:Pr) ratio did not differ among treatments ($P_{Trt} \geq 0.19$).

Diurnal rumen pH was affected by treatments ($P_{Trt} = 0.02$; Table 7) and was higher for HDcon than other treatments. The diurnal pH varied ($P_{Time} < 0.01$; Figure 2) and was lowest after night feeding. Time below pH 5.8 and pH 5.6 was longer when cows were fed extruded pellets than conventional pellets ($P_{Con \times Ext} \leq 0.05$).

Rumen pool of starch was tended to be higher for HDext than LDext treatment ($P_{Trt} = 0.07$; Table 8). Passage rate, as well as digestion rate, of starch did not differ among treatments ($P_{Trt} \geq 0.24$), but the digestion rate of DM for conventional pellets was higher than extruded pellets ($P_{Con \times Ext} = 0.01$).

3. 5. The postprandial duodenal flow of dry matter, starch, and protein

On postprandial sampling day, total DM, starch, and CP intake did not differ among treatments ($P_{Trt} \geq 0.13$; Table 9), but silage intake was higher for conventional pellets than for extruded pellets ($P_{Con \times Ext} = 0.01$).

Concerning the first postprandial sequence, the postprandial DM flow increased after feeding for all treatments ($P_{Time} < 0.01$; Table 9), and this increase tended to be higher for LDext compared with MDext and HDcon treatments up to 8h after morning feeding ($P_{Time \times Trt} = 0.07$; Figure 3). When taking the total time period into consideration, DM flow was not affected by treatments ($P_{Trt} = 0.13$) but tended to be higher for extruded pellets than conventional pellets ($P_{Con \times Ext} = 0.09$).

Postprandial starch flow did not differ among treatments ($P_{Trt} \geq 0.14$; Table 9) but was higher for high-density pellets than other density pellets ($P_{HD \times LMD} \leq 0.04$). As for DM, postprandial starch flow increased after feeding for all treatments ($P_{Time} < 0.01$) and, compared with LDext, MDext, and HDext treatments, the increase was highest for HDcon at first 3 h but then was overtaken by HDext pellets ($P_{Time \times Trt} \leq 0.08$; Figure 4A). Similarly, postprandial starch concentration in duodenal digesta increased after feeding ($P_{Time} < 0.01$) and was higher for high-density pellets than for other density pellets ($P_{HD \times LMD} = 0.03$; Figure 4B).

Postprandial CP flow differed among treatments ($P_{Trt} \leq 0.03$; Table 9) and was higher for LDext than for other treatments. The CP concentration in the duodenal digesta was higher for LDext and HDcon than other treatments ($P_{Trt} \leq 0.05$). CP flow increased after feeding for all treatments ($P_{Time} \leq 0.01$; Figure 5A), whereas the concentration of CP in duodenal digesta increased slightly throughout the day towards the evening ($P_{Time} \leq 0.02$; Figure 5B).

3.6. In situ degradability

The ESD differed among the treatments ($P_{Trt} \leq 0.04$; Table 10), and it was lower for HDext than HDcon treatment. The main explanation was a lower *S* fraction and corresponding higher *Pd* fraction in HDext than HDcon treatment ($P_{Trt} < 0.01$). The k_d of starch did not differ among treatments ($P_{Trt} = 0.22$) and between conventional and extruded pellets ($P_{Con \times Ext} = 0.58$). The *in situ* rumen escape of starch, based on ESD calculated with constant passage rates, did not differ among treatments ($P_{Trt} \geq 0.23$).

The EPD was lowest for LDext and highest for HDext pellets ($P_{Trt} < 0\ .01$; Table 10). The main explanation was differences in k_d of protein among treatments which was lowest for LDext followed by HDcon, MDext, and HDext, all being different ($P_{Trt} < 0.01$). Conversely, the

calculated *in situ* rumen escape of protein was higher for LDext and HDcon pellets than for MDext and HDext ($P_{Trt} < 0.01$). However, estimated duodenal microbial protein flow did not differ among treatments ($P_{Trt} \geq 0.12$) but was higher for extruded pellets than for conventional pellets ($P_{Con \times Ext} \leq 0.04$).

3.7. Milk production and N balance

Daily production of milk, milk constituents, and ECM did not differ among treatments ($P_{Trt} \geq 0.15$; Table 11), but ECM produced per kg concentrate consumed tended to be higher for HDext than for LDext ($P_{Trt} = 0.09$) and for high-density pellets than other density pellets ($P_{HD \times LMD} = 0.07$). The protein concentration in milk was higher for conventional pellets than for extruded pellets ($P_{Con \times Ext} = 0.04$). Somatic cell count was higher for HDcon than for LDext and MDext treatments ($P_{Trt} = 0.03$) and conventional pellets than extruded pellets ($P = 0.01$).

On average, 88.7% of ingested N was recovered in feces, urine, and milk with no difference among treatments ($P_{Trt} = 0.95$; Table 12). Around 37, 30, and 33% of excreted N were excreted in feces, urine, and milk, respectively, with no differences among treatments ($P_{Trt} \geq 0.47$).

4. Discussion

Khan et al. (2021b) investigated extruded pellets of pure barley differing in physical properties and found that high density combined with high fluid stability of extruded pellets could reduce RSD by facilitating a decrease in the rate of degradation combined with an increase in the rate of passage. Thus, an objective of the current study was to compare these pellets with conventional pellets. As expected, conventional pellets showed high density; however, FSI was low and agrees with Larsen and Raun (2018), who observed lower water stability in 24

commercial pelletized concentrates for dairy cows. On the other hand, three extruded pellets used had different densities depending upon operating settings in the extruder, but all had high FSI. Higher fluid stability of pellets is closely linked to enhanced physical integrity and reduced disintegration (Welker et al., 2018) and hence crucial for maintaining density properties in pellets in the rumen. The current experiment was conducted using pellets made from 70% barley and 30% SBM. In contrast to an earlier experiment studying pellets of 50% barley and 50% SBM (Khan et al., 2021a), the density and fluid stability of extruded pellets were demonstrated to be within the range expected to affect rumen escape.

4.1. Rumen digestion and outflow

4.1.1. Starch

Using expander pelleting, Prestløkken and Harstad (2001) and Tothi et al. (2003) observed an RSD of 91% for barley, about 4% unit higher than observed for HDcon in the present study. However, for both HDext and HDcon, an RSD of 87% was almost equal to the average RSD reported for barley (Nocek and Tamminga, 1991; Moharrery et al., 2014). Typically, heat processing results in starch gelatinization and an increased degree of starch digestion. In expander processing, 22 to 35% starch gelatinization is expected, whereas, in extruder cooking, gelatinization can be up to 100% due to high moisture, temperature, pressure, and shear (Svihus et al., 2005). Thus, high RSD by extruder processing was expected, but it did not differ from conventional pelleting. Tothi et al. (2003) found no differences in RSD between ground and expander pelleted barley. Similarly, Ljøkjel et al. (2003) found that neither ordinary (81 °C) pelleting nor expander (110 °C or 128 °C) pelleting had significant effects on *in situ* degradation of starch in barley and wheat. This was confirmed *in vivo* by Prestløkken and Harstad (2001), who observed no significant differences in RSD between ordinary (75-80 °C) pelleted and

496 expander (125-130 °C) pelleted barley-based diet, although expanding numerically increased
497 RSD (91 *versus* 86%). The lack of a clear response of heat treatment on RSD for barley and
498 wheat could be attributed to their already high ruminal degradability. However, intense heat
499 treatment has even been reported to reduce ESD, particularly in readily degradable starch
500 sources (Offner et al., 2003; Razzaghi et al., 2016). A possible explanation put forward has been
501 heat treated protein protecting starch granules from rumen digestion (Svihus et al., 2005). Thus,
502 current differences in RSD between treatments are most likely due to the effects of physical
503 properties of feed pellets.

504 Despite marked differences in fluid stability, the RSD of HDext did not differ from HDcon. This
505 was surprising because it was expected that HDext would have lower starch k_d and higher starch
506 k_p owing to its high fluid stability and high density, allowing pellets to degrade slowly and sink
507 into reticulum or/and ventral rumen, resulting in the greater likelihood of rumen escape.
508 Although k_d of starch determined *in situ* (Table 10) and *in vivo* (Table 8) did not differ among
509 treatments, both methods provided similar patterns where k_d of HDext was numerically lower
510 than the other treatments. On the other hand, the specific density of HDext after soaking in
511 rumen fluid for 20 min was 1.2 g/mL. This density was within the optimal range of 1.2 to 1.3
512 g/mL, as suggested by Dufreneix et al. (2019), for feed particles to promote passage from the
513 reticulorumen. However, a density range of 1.17 to 1.40 g/mL had been observed to have
514 increased passage from the reticulorumen using inert plastic particles (desBordes and Welch,
515 1984; Seyama et al., 2017). In contrast to HDext, HDcon pellets were expected to disintegrate
516 rapidly in the rumen due to lower FSI, losing the density properties. Therefore, the similar RSD
517 between HDcon and HDext was quite intriguing. Moreover, HDcon was expected to induce
518 local acidic conditions in the ventral rumen as conventional pellets, even though low in starch,

had been observed to reduce pH in the ventral rumen (Khafipour et al., 2009). This pH reduction for conventional pellets is assumed to be caused by the increased degradation rate due to quick pellet disintegration. However, the current patterns of fermentation variables in the ventral rumen (Table 6) do not support this. When the specific density of HDcon in rumen fluid was determined after soaking for 10 min to avoid pellet disintegration, its specific density (1.17 g/mL) was close to the optimal range suggested for increased outflow from the rumen. Hence, most pellets of HDcon, despite having low FSI, were quickly attaining the required density allowing rumen escape before being completely disintegrated. However, *in situ* determination revealed that HDcon had a higher soluble fraction compared with HDext. Soluble fraction is supposed to be caused mainly by the small particles' loss through the bag pores (Hvelplund and Weisbjerg, 2000); yet, it is considered immediately degradable in the rumen. These particles may have k_d like the particles remaining in the bag (de Jonge et al., 2015) and/or may escape the rumen with the liquid phase having higher *kp* (0.09 h^{-1} in the current experiment). In routine, samples are ground prior to rumen incubation in the *in situ* procedure. However, to better observe the effects of pellets' physical properties on rumen degradation kinetics, we used intact pellets. Moreover, correction with initial loss of small particles was not conducted. The observed high soluble fraction in HDcon was probably because of increased loss of small particles due to its low FSI. These small particles might have left the rumen faster than being degraded in the rumen, and this may explain why *in situ* ESD and *in vivo* RSD for HDcon were better related when ESD was estimated with *kp* of 0.08 h^{-1}. Thus, HDcon might had higher k_d but also a higher k_p than HDext, giving similar RSD.

These explanations are supported by the postprandial duodenal starch flow patterns, even though 100% experimental treatments were used during these measurements. Although similar mean

duodenal starch appearance, HDcon appeared to have a more rapid rumen outflow than HDext (Figure 4A). Rumen outflow of undegraded starch is generally assumed to follow first-order kinetics with an exponential decline. This assumption fits well for HDcon. However, rumen outflow of starch with HDext, and extruded pellets in general, indicates a lag time of newly ingested starch before passage, challenging the assumption of first-order kinetics. Probably, HDext took a longer time to attain the necessary density to escape rumen than HDcon. On the other hand, a quick increase in specific density and a faster passage for small detached particles were the factors giving a rapid rumen outflow for HDcon than for HDext and hence a similar duodenal starch appearance. Our findings also contradict Larsen et al. (2019), who suggested that feed pellets with high density and fluid stability could have higher rumen escape. Although DMI did not differ among treatments, the eating behavior for HDext varied among the cows, which can influence the outflow from the rumen. It seems that some cows had problems eating these pellets as reflected by higher hardness (with knife knob) than other treatments. Thus, if all cows were eating similarly, there could have been differences in the starch outflow from the rumen between HDext and HDcon.

Both MDext and LDext had low specific density in rumen fluid (1.05 and 0.85 g/mL, respectively) and, thus, a low likelihood of escaping the rumen, resulting in high RSD. High RSD usually results in a pH drop due to increased VFA production. Moreover, an increased proportion of propionate and decreased Ac:Pr ratio in rumen fluid is usually observed (Overton et al., 1995). Lower pH and a higher propionate concentration in dorsal rumen fluid for LDext than for HDcon and HDext indicate reduced passage and high fermentation of LDext in dorsal rumen owing to its low density and floating behavior.

Unfortunately, we could not observe any differences in starch k_p among treatments by the rumen evacuations; however, differences were somehow reflected in starch pool sizes. Calculation of k_p assumes first-order digestion kinetics and steady-state conditions and that the rumen pool obtained from evacuations is representative for the mean rumen pool size (Huhtanen and Sveinbjörnsson, 2006). By analyzing the data, it was revealed that rumen starch pool sizes for LDext and MDext in period 4 were smaller than expected. When these two values were removed, k_p of starch for high-density pellets became significantly higher than other density pellets ($P_{HD \times LMD} = 0.05$). A k_p of 0.034 h^{-1}, achieved for LDext and MDext, corresponded well with RSD and postprandial starch flow. Huhtanen and Sveinbjörnsson (2006) suggested more frequent rumen evacuations and careful selections of rumen evacuation times to reduce diurnal variation in rumen starch pool. By conducting a series of rumen evacuations after pulse dosing concentrate once daily and correcting the rumen starch pools with the corresponding duodenal starch flow (g/h) at individual times, Tothi et al. (2003) showed diurnal variation in k_p of starch. Hence, the assumption of simple first-order kinetics for starch passage is equivocal, as supported by postprandial starch flow in the present study. Adding more rumen evacuations probably would have benefitted our study, increasing the probability of finding differences in k_p among treatments.

Similar k_d, particularly for *in situ* determinations, for LDext and HDcon, was also unexpected based on the differences in FSI. This discrepancy was probably due to the missing of 2 h incubation. When FSI was determined after 120 min of incubation, the FSI of LDext was considerably reduced (data not shown). It can be speculated that k_d might have been different between these two treatments during the first two hours in the rumen, but the differences subsequently diminished due to the complete disintegration of LDext pellets. Moreover, a

similar trend in k_d during *in vivo* indicates that perhaps the effect of physical forces (e.g., by motility and digesta contents) during *in situ* and *in vivo* was enormous on feed pellet disintegration, thereby increasing microbial digestion access than *in vitro* determination of FSI. Moreover, rumen pH was lower for extruded pellets, particularly for LDext and MDext, than conventional pellets. Extruded pellets were assumed to degrade more slowly due to their high FSI, thereby providing higher rumen pH. However, time spent below 5.6 and 5.8 were all less than suggested for a 24-h period (Khafipour et al., 2009; Zebeli and Metzler-Zebeli, 2012), indicating a low possibility for developing sub-acute ruminal acidosis (SARA) (Krause and Oetzel, 2006) with extruded pellets.

4.1.2. Protein

Based on rumen escape of starch, a greater rumen outflow of dietary CP could be expected for high-density pellets, especially for HDext, than other density pellets. However, the ruminal outflow of CP appeared to increase linearly from high- to low-density pellets. Increased rumen outflow for LDext fit with the lower *in situ* EPD observed, pointing towards a higher rumen escape of dietary CP. Moreover, ammonia and branched-chained fatty acids such as isobutyric and isovaleric acid are the product of rumen deamination of amino acids (Cunningham and Klein, 2013), and a decreased concentration of particularly isobutyric acid has been reported when cows were fed low rumen degradable proteins (Reynal and Broderick, 2003; Colmenero and Broderick, 2006). Thus, a lower concentration of isobutyric acid in rumen fluid for LDext may support decreased rumen protein degradation, although ammonia and isovalerate concentrations did not differ.

The increase in duodenal CP flow could also be attributed to the increased synthesis of microbial crude protein (MCP) in the rumen. MCP synthesis depends on the rate of carbohydrate

610 fermentation and sequestration of ammonia and preformed amino acids, and these are positively
611 correlated (Nocek and Russell, 1988). Overall ruminal carbohydrate digestion did not differ
612 among treatments (data not shown). Therefore, despite low ruminal pH and fiber digestion
613 (discussed below), this could indicate a positive effect on MCP synthesis through
614 synchronization of rumen release of nutrients (Herrera-Saldana et al., 1990; Elseed, 2005) with
615 extruded pellets, particularly for LDext. Thus, unlike starch, it seems that the rumen metabolism
616 of protein was affected mainly by heat treatment rather than pellets' physical properties.
617 However, due to the complexity of rumen synchronization (Yang et al., 2010) and lack of actual
618 estimates of rumen escape of dietary protein and duodenal flow of microbial protein, this needs
619 proper investigations.

620 *4.2. Post-rumen digestion of starch and protein*

621 To achieve increased energy supply through glucose absorption, starch entering the duodenum
622 must be digested in the small intestine (Huntington et al., 2006; Owens et al., 2016). It has been
623 demonstrated that ruminal and small intestinal starch digestibility are positively correlated
624 (Nocek and Tamminga, 1991), and the efficiency of small intestinal starch digestion has been
625 observed to vary with starch sources and feed processing (Owens et al., 1986). Total tract
626 digestion of starch exceeded 99% in all treatments, indicating high post ruminal digestion of
627 starch. The observed ileal digestibility of more than 98% indicated no negative impact on small
628 intestinal starch digestibility and consequent hindgut fermentation; indeed, this was based on
629 only two cows with ileal cannulas. Thus, taking RSD into consideration, high-density pellets,
630 particularly HDext, resulted in a higher small intestinal absorption of glucose from starch than
631 the other density pellets.

632 The observed higher post-ruminal digestion of protein as a percentage of intake for extruded
633 pellets, particularly for LDext, indicates a greater supply of metabolizable protein than
634 conventional pellets. However, the daily amount of protein digested post-ruminally was not
635 different among treatments (P_{Trt}= 0.17; data not shown), although patterns of digestion
636 numerically were the same as for duodenal flow, i.e., being greater for extruded than
637 conventional pellets. This, and a similar apparent total tract digestibility of CP among
638 treatments, demonstrate that differences may have been masked by a high hindgut fermentation
639 of OM for extruded pellets, thereby increasing microbial growth and loss of MCP in the feces.

640 *4.3. Effects on fiber digestion*

641 Allocation of rapidly degradable starch has been observed to decrease the ruminal and total tract
642 digestibility of fiber (McCarthy et al., 1989; Overton et al., 1995; Chibisa et al., 2015). Thus, an
643 apparent lower NDF digestion for extruded pellets contrasted our expectation as slow
644 degradation of starch in these pellets was assumed to increase NDF digestion compared with
645 conventional pellets. However, the low ruminal digestion for NDF was compensated by hindgut
646 fermentation eliminating differences in total tract digestion of NDF among treatments. The
647 activity of cellulolytic bacteria is sensitive to changes in pH, and a pH below 6 is recognized to
648 impair the growth of these bacteria (Russell and Wilson, 1996). Apparently, an overall lowered
649 rumen pH for extruded pellets (Table 6 and 7) may explain lower ruminal NDF digestion for
650 these pellets. However, the corresponding increased protein flow does not support this, which
651 may indicate high MCP synthesis. There might be some other unknown conditions in the rumen
652 that are unfavorable for fiber digestion, altering the site of NDF digestion in extruded pellets.

653 *4.4. Effects on milk production and composition*

Except for protein concentration and somatic cell count, no significant effects on milk production were found. However, a high ECM produced per kg concentrate consumed, particularly for HDext, indicates increased nutrient supply for milk production. Increased post-ruminal starch digestion has been reported to increase milk yield but decrease milk fat concentration resulting in similar ECM yields (Reynolds, 2006). Compared to ordinary pelleting, expander pelleting has been reported to increase milk yield and milk protein content (Prestløkken and Harstad, 2001), indicating increased absorption of amino acids from the small intestine. In the present study, despite higher intestinal digestibility of protein, milk protein contents were low for extruded pellets. However, it must be emphasized that the findings are based on a limited amount of data.

5. Conclusion

The current study demonstrated that the physical properties of feed pellets affect ruminal digestion kinetics, particularly of starch. High-density pellets appeared to have greater rumen outflow and thereby lower RSD than other density pellets. However, the study did not provide clear support for the hypothesis of increased rumen escape with high-density extruded pellets having high fluid stability. Although our findings negate the importance of fluid stability on digestion kinetics, feed pellets with certain FSI will indeed be required to exhibit the effect of density on passage from the reticulorumen. Clearly, more research is needed regarding the interaction of density and fluid stability of feed pellets on nutrient digestion kinetics in the rumen.

Conflict of interest statement

None of the authors have conflicts of interest

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Table 1

Chemical composition (g/kg DM if not stated otherwise) and physical properties of experimental treatments

Item	Experimental treatments[1]			
	HDcon	HDext	MDext	LDext
Chemical Composition				
Dry matter (DM), g/kg	902	866	886	898
Crude protein	232	225	221	233
Starch	428	425	422	406
aNDFom	174	160	175	192
WSC[2]	46	46	47	46
Ash	33	32	32	35
Fat	9.8	4.2	2.5	3.6
Physical Properties				
Radial expansion, %	0	38	51	62
Bulk density, g/L	670	650	545	410
Specific density, g/mL				
Dry pellets	1.11	1.07	0.87	0.66
Wet pellets[3]	1.17	1.20	1.05	0.85
Sinking velocity, mm/sec	120	100	25	-
Hardness, N				
Flat knob	164	135	72	55
Knife knob	48	150	96	50

[1] HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets.

[2] WSC= Water soluble carbohydrates

[3] Determined after soaking pellets in rumen fluid for 20 min at 39 °C. HDcon was soaked for 10 min due to pellet disintegration.

Table 2

Chemical composition of grass silage and commercial compound (g/kg DM, if not stated otherwise).

Item	Grass silage[1,2]	Commercial compound[3]
DM, g/kg	264	888
Crude protein	112	194
Starch	-	348
Crude Fat	34	54
aNDFom	510	214
WSC[4]	113	64
Ash	56	62

[1] Mixed blend of two types (see text for details), *in vitro* digestible organic matter (OM), 698 g/kg OM (Eurofins Agro Testing Norway AS, NO-1538 Moss)

[2] Multimineral mix (Pluss Storfe multitilskudd, Felleskjøpet, Agri, Lillestrøm, Norway) spread over containing (per kg): 95 g of Ca, 55 g of P, 90 g of Mg, 95 g of Na, 127 g of Cl, 1 g of K, 400 kIU of vitamin A, 120 kIU of vitamin D, 3000 mg of vitamin E, 100 mg of biotin, 1200 mg of Cu, 150 mg of I, 20 mg of Co, 3000 mg of Mn, 4000 mg of Zn, 25 mg of Se.

[3] FORMEL Favør 80, Felleskjøpet Agri, Lillestrøm, Norway

[4] WSC= Water soluble carbohydrates

Table 3

Nutrient intake (kg/day)

Item	Experimental treatments[1]				SEM[2]	*P*-Value		
	HDcon	HDext	MDext	LDext		Trt	HD × LMD	Con × Ext
Dry matter (DM)								
Experimental feed	6.19	5.77	6.14	6.21	0.14	0.18	0.21	0.38
Commercial compound[3]	2.66	2.44	2.63	2.66	0.07	0.15	0.19	0.34
Silage	12.5	13.4	12.2	12.4	1.55	0.13	0.10	0.61
Total	21.3	21.6	21.0	21.3	1.59	0.82	0.48	0.93
Organic matter (OM)	20.3	20.5	20.0	20.2	1.50	0.83	0.48	0.92
Starch	3.58	3.30	3.51	3.45	0.11	0.38	0.74	0.24
Crude Protein	3.35	3.26	3.23	3.35	0.18	0.48	0.85	0.41
aNDFom	8.00	8.26	7.88	8.08	0.80	0.56	0.44	0.75
WSC[4]	1.88	1.92	1.82	1.86	0.18	0.52	0.22	0.77
Ash	1.06	1.08	1.04	1.07	0.09	0.56	0.50	0.97
Fat	0.63[a]	0.60[ab]	0.56[b]	0.58[b]	0.05	0.05	0.02	0.02

[1] HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets.

[2] SEM= Standard error of the mean for n=4

[3] FORMEL Favør 80, Felleskjøpet Agri, Lillestrøm, Norway

[4] WSC= Water soluble carbohydrates

[a, b] indicate least-square means to differ within the row

Table 4

Duodenal flow and apparent digestibility of starch and crude protein (CP) along the gastrointestinal tract

Item	Experimental treatments[1]				SEM[2]	*P*-value		
	HDcon	HDext	MDext	LDext		Trt	HD × LMD	Con × Ext
Starch								
Duodenal flow, kg/d	0.43^{ab}	0.45^{a}	0.36^{bc}	0.33^{c}	0.05	0.09	0.02	0.22
Digestibility								
Rumen, % of intake	88.0^{bc}	86.4^{c}	89.9^{ab}	90.5^{a}	1.16	0.02	0.01	0.29
Intestinal								
% of entering	98.4^{ab}	99.6^{a}	97.6^{ab}	96.4^{b}	0.80	0.07	0.03	0.56
% of intake	11.8^{ab}	13.5^{a}	9.99^{bc}	9.12^{c}	1.12	0.02	0.01	0.28
Ileal[3], % of intake	98.5	98.7	98.4	98.5	0.33	0.97	0.78	0.98
Total tract, % of intake	99.8^{ab}	99.9^{a}	99.8^{ab}	99.6^{b}	0.09	0.06	0.03	0.80
Crude protein								
Duodenal flow, kg/d	4.39	4.56	4.59	4.90	0.34	0.15	0.08	0.10
Digestibility								
Rumen, % of intake	-30.9^{c}	-39.0^{b}	-41.8^{ab}	-45.9^{a}	2.87	<0.01	<0.01	<0.01
Intestinal								
% of entering	75.1	76.3	77.2	76.5	1.00	0.12	0.06	0.04
% of intake	98^{c}	106^{b}	110^{a}	112^{a}	2.28	<0.01	<0.01	<0.01
Ileal[3], % of intake	61.6	64.7	59.4	60.6	5.92	0.94	0.72	0.99
Total tract, % of intake	67.4	67.0	67.9	66.0	1.72	0.62	0.79	0.72

[1] HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets.

[2] Standard error of the mean for n = 4

[3] Digestibility up to distal ileum with SEM for n = 2

[a, b, c, d] indicate least-square means to differ within the row

Table 5

Duodenal flow and apparent digestibility of DM, organic matter (OM), and aNDFom along the gastrointestinal tract

Item	Experimental treatments[1]				SEM[2]	*P*-value		
	HDcon	HDext	MDext	LDext		Trt	HD × LMD	Con × Ext
DM								
Duodenal flow, kg/d	15.7	17.0	16.3	17.2	1.60	0.17	0.36	0.07
Digestibility								
Rumen, % of intake	26.6^{a}	22.0^{b}	22.8^{b}	19.3^{c}	1.71	<0.01	0.01	<.01
Intestinal								
% of entering	57.1^{b}	59.5^{a}	60.6^{a}	60.5^{a}	0.88	0.01	<0.01	<0.01
% of intake	42^{c}	46.5^{b}	47.0^{ab}	49.2^{a}	1.36	<0.01	<0.01	<0.01
Total tract, % of intake	68.6^{b}	68.5^{b}	69.7^{a}	68.4^{b}	0.87	0.05	0.11	0.35
OM								
Duodenal flow, kg/d	12.8	13.9	13.1	14.1	1.30	0.16	0.54	0.09
Digestibility								
Rumen, % of intake	36.9^{a}	32.6^{bc}	34.5^{ab}	30.6^{c}	1.61	0.02	0.06	0.01
Intestinal								
% of entering	52.2^{b}	55.0^{ab}	55.6^{a}	56.1^{a}	1.12	0.06	0.04	0.01
% of intake	33.0^{b}	37.3^{a}	36.5^{a}	39.2^{a}	1.34	0.02	0.03	0.01
Total tract, % of intake	69.9^{b}	69.9^{b}	71.1^{a}	69.8^{b}	0.82	0.06	0.10	0.34
aNDFom								
Duodenal flow, kg/d	4.13	4.62	4.22	4.76	0.60	0.26	0.64	0.19
Digestibility								
Rumen, % of intake	48.7	44.9	46.6	41.4	2.69	0.11	0.16	0.07
Intestinal								
% of entering	3.49^{b}	10.8^{ab}	11.0^{ab}	18.6^{a}	3.58	0.06	0.04	0.03
% of intake	2.1^{b}	7.0^{ab}	6.02^{ab}	11.2^{a}	2.22	0.06	0.06	0.03
Total tract, % of intake	50.8	52.0	52.7	52.6	1.22	0.43	0.18	0.15

[1] HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets.

[2] SEM= Standard error of the mean for n=4

[a, b, c] indicate least-square means to differ within the row

Table 6

Postprandial rumen pH and VFA patterns

Item	Experimental treatments[1]				SEM[2]	*P*-Values				
	HDcon	HDext	MDext	LDext		Trt	Time	Time × Trt	HD × LMD	Con × Ext
Dorsal										
pH	6.42[a]	6.38[a]	6.31[b]	6.30[b]	0.05	0.01	<0.01	0.52	<0.01	0.01
Total VFA, mM/L	87	87.4	91.4	89	2.21	0.49	<0.01	0.72	0.19	0.39
Acetate, % of tot	65.3	65.7	65.8	65.2	0.48	0.69	<0.01	0.68	0.98	0.66
Propionate, % of tot	17.6[b]	17.9[b]	17.6[b]	18.8[a]	0.54	0.05	<0.01	0.73	0.16	0.19
Butyrate, % of tot	13.9[a]	13.2[ab]	13.3[ab]	12.9[b]	0.59	0.06	<0.01	0.99	0.06	0.01
Isobutyrate, % of tot	0.70[a]	0.70[a]	0.71[a]	0.64[b]	0.03	0.02	<0.01	0.69	0.18	0.43
Valerate, % of tot	1.33	1.30	1.35	1.35	0.04	0.74	<0.01	0.96	0.37	0.99
Isovalerate, % of tot	1.08	1.15	1.17	1.08	0.12	0.73	<0.01	0.88	0.93	0.56
Acetate:Propionate	3.77	3.76	3.81	3.51	0.14	0.19	<0.01	0.64	0.33	0.53
Ammonia, mg/L	88.5	84.3	77.6	82.9	11.7	0.86	<0.01	0.88	0.50	0.52
Medial										
pH	6.24	6.13	6.13	6.17	0.05	0.15	<0.01	0.98	0.36	0.04
Total VFA, mM/L	97.4	99.6	102.3	98	2.43	0.45	<0.01	0.64	0.48	0.34
Acetate, % of tot	65.2	65.4	65.4	64.9	0.50	0.83	<0.01	0.98	0.75	0.92
Propionate, % of tot	17.6	18.1	17.9	18.6	0.51	0.29	<0.01	0.98	0.24	0.17
Butyrate, % of tot	14	13.3	13.4	13.3	0.64	0.34	<0.01	0.99	0.29	0.08
Isobutyrate, % of tot	0.70[a]	0.67[ab]	0.70[a]	0.64[b]	0.03	0.04	<0.01	0.64	0.26	0.10
Valerate, % of tot	1.39	1.34	1.39	1.43	0.05	0.53	<0.01	0.97	0.31	0.95
Isovalerate, % of tot	1.09	1.14	1.19	1.10	0.13	0.80	<0.01	0.93	0.77	0.60
Acetate:Propionate	3.76	3.67	3.70	3.53	0.13	0.57	<0.01	0.97	0.40	0.35
Ammonia, mg/L	80.3	79.8	72.9	72.4	10.4	0.87	<0.01	0.93	0.42	0.61
Ventral										
pH	6.44	6.45	6.45	6.47	0.04	0.92	<0.01	0.66	0.63	0.59
Total VFA, mM/L	90.6	89.2	91.7	91.5	1.79	0.71	<0.01	0.43	0.31	0.90
Acetate, % of tot	65	65.8	65.7	65.5	0.47	0.61	<0.01	0.81	0.67	0.22
Propionate, % of tot	17.8	17.9	17.9	18.6	0.52	0.42	<0.01	0.94	0.30	0.40
Butyrate, % of tot	14[a]	13.2[ab]	13.2[ab]	13[b]	0.57	0.09	0.01	0.23	0.08	0.02
Isobutyrate, % of tot	0.75[a]	0.71[a]	0.72[a]	0.64[b]	0.04	0.01	<0.01	0.89	0.01	0.01
Valerate, % of tot	1.35	1.27	1.32	1.31	0.05	0.55	<0.01	0.36	0.84	0.30
Isovalerate, % of tot	1.22	1.13	1.18	1.06	0.12	0.55	<0.01	0.74	0.97	0.97
Acetate:Propionate	3.70	3.73	3.73	3.56	0.13	0.64	<0.01	0.83	0.50	0.77
Ammonia, mg/L	82.2	80.1	74	76	11.4	0.87	<0.01	0.99	0.44	0.54

[1] HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets.

[2] SEM= Standard error of the mean for n=4

[a, b] indicate least-square means to differ within the row

Table 7

Diurnal rumen pH and time spent below a certain pH point over a 24 h period

Item	Experimental treatments[1]				SEM[2]	*P*-Values				
	HDcon	HDext	MDext	LDext		Trt	Time	Time × Trt	HD × LMD	Con × Ext
pH	6.12[a]	6.05[b]	6.04[b]	6.01[b]	0.05	0.02	<0.01	0.80	0.01	<0.01
pH < 6.4, h/d	21.2	22.4	21.6	22.2	1.18	0.82	-	-	0.95	0.49
pH < 6.2, h/d	15.0	16.8	17.8	18.5	1.70	0.39	-	-	0.16	0.14
pH < 6.0, h/d	7.23	9.80	10.3	10.7	2.32	0.34	-	-	0.19	0.10
pH < 5.8, h/d	1.88	4.34	4.54	4.75	1.50	0.22	-	-	0.16	0.05
pH < 5.6, h/d	0.25	1.22	1.50	1.70	0.59	0.14	-	-	0.07	0.04

[1] HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets.

[2] SEM= Standard error of the mean for n=4

[a, b] indicate least-square means to differ within the row

Table 8

Rumen pool size, passage and digestion rates of starch and dry matter (DM)

Item	Experimental treatments[1]				SEM[2]	*P*-Value		
	HDcon	HDext	MDext	LDext		Trt	HD × LMD	Con × Ext
Rumen volume, kg	96	102	101	103	7.39	0.49	0.34	0.16
Rumen pool, kg								
Starch	0.381[ab]	0.434[a]	0.397[ab]	0.345[b]	0.028	0.07	0.10	0.62
DM	12.6	13.6	12.8	13.7	1.11	0.32	0.79	0.23
Passage rate, h^{-1}								
Starch	0.047	0.044	0.040	0.044	0.007	0.87	0.61	0.54
DM	0.052	0.052	0.054	0.052	0.004	0.92	0.62	0.73
Liquid	0.097	0.091	0.085	0.091	0.005	0.44	0.24	0.20
Digestion rate, h^{-1}								
Starch	0.345	0.274	0.342	0.429	0.049	0.24	0.16	0.95
DM	0.019[a]	0.015[b]	0.016[ab]	0.012[b]	0.002	0.02	0.05	0.01

[1] HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets.

[2] SEM= Standard error of the mean for n=4

[a, b] indicate least-square means to differ within the row

Table 9

Intakes and postprandial duodenal flow of dry matter (DM), starch, and crude protein (CP)

Item	Experimental treatments[1]				SEM[3]	*P* values				
	HDcon	HDext[2]	MDext	LDext		Trt	Time	Time × Trt	HD × LMD	Con × Ext
Intake:										
Concentrate, kg/d	7.73	7.88	6.90	8.88	1.00	0.29	-	-	0.91	0.84
Silage, kg/d	12.5^{a}	11.0^{b}	11.0^{b}	10.6^{b}	1.36	0.04	-	-	0.06	0.01
Total DM, kg/d	20.2	18.8	17.9	19.5	1.82	0.13	-	-	0.23	0.08
Starch, kg/d	3.33	3.41	2.92	3.61	0.47	0.45	-	-	0.75	0.95
CP, kg/d	3.20	3.00	2.75	3.26	0.30	0.14	-	-	0.56	0.29
Up to 8h after morning feeding										
DM, g/h	889^{b}	944^{ab}	911^{b}	1029^{a}	81	0.10	<0.01	0.07	0.23	0.14
Starch, g/h	37.4	36.8	19.1	24.0	6.00	0.14^{T}	<0.01^{T}	0.06^{T}	0.03^{T}	0.15^{T}
Starch. g/kg DM	41.1^{a}	35.2^{ab}	20.0^{b}	22.9^{b}	6.24	0.07^{T}	<0.01^{T}	0.34^{T}	0.03^{T}	0.06^{T}
CP, g/h	246^{b}	242^{b}	232^{b}	281^{a}	17.3	0.03	<0.01	0.24	0.28	0.65
CP, g/kg DM	281^{a}	263^{ab}	261^{b}	279^{a}	7.50	0.05	0.02	0.42	0.75	0.07
Up to 17h after morning feeding										
DM, g/h	878	938	907	976	5.00	0.13	<0.01	0.24	0.331	0.09
Starch, g/h	34.9	36.6	20.4	26.9	5.00	0.13^{T}	<0.01^{T}	0.08^{T}	0.04^{T}	0.29^{T}
Starch. g/kg DM	37.7^{a}	37.6^{a}	21.5^{b}	27.4^{ab}	4.84	0.10^{T}	<0.01^{T}	0.38^{T}	0.03^{T}	0.16^{T}
17 h bypass starch, %	20	20	12	13	3.00	0.12	-	-	0.03	0.12
CP, g/h	244^{b}	254^{ab}	240^{b}	281^{a}	17.7	<0.01	<0.01	0.61	0.17	0.11
CP, g/kg DM	282^{ab}	275^{bc}	270^{c}	291^{a}	6.90	<0.01	<0.01	0.17	0.59	0.48

[1] HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets.

[2] Only data from three cows on the HDext treatment

[3] SEM= Standard error of the mean for n=4

[T] *P* values are for the square root transformed variable.

[a, b, c] indicate least-square means to differ within the row

Table 10

In situ rumen degradation of starch and crude protein (CP)

Item[2]	Experimental treatments[1]				SEM[3]	*P*-value		
	HDcon	HDext	MDext	LDext		Trt	HD × LMD	Con × Ext
Starch								
S, %	24.9[a]	3.6[d]	5.5[c]	13.3[b]	0.24	<0.01	<0.01	<0.01
Pd, %	73.9[d]	95.9[a]	93.8[b]	86.1[c]	0.41	<0.01	<0.01	<0.01
D, %	98.9	99.5	99.2	99.4	0.22	0.28	0.67	0.09
k_d, %	44.2	36.4	43.7	46.3	3.24	0.22	0.18	0.58
ESD_5, %	91.4[a]	87.0[b]	88.3[ab]	90.4[ab]	0.95	0.04	0.86	0.03
ESD_8, %	87.6[a]	81.0[b]	83.1[ab]	85.9[ab]	1.36	0.03	0.89	0.03
In situ rumen escape by ESD_5, kg/d	0.34	0.42	0.41	0.35	0.04	0.24	0.88	0.15
In situ rumen escape by ESD_8, kg/d	0.48	0.60	0.58	0.50	0.06	0.23	0.90	0.15
Crude Protein								
S, %	1.05[b]	2.90[a]	3.70[a]	0.00[b]	0.6	0.01	0.77	0.15
Pd, %	99.0[ab]	97.1[b]	96.3[b]	100[a]	0.6	0.01	0.77	0.15
D, %	100	100	100	100	-	-	-	-
k_d, %	5.6[c]	6.8[a]	6.2[b]	4.9[d]	0.14	<0.01	<0.01	0.04
EPD_5, %	53.1[c]	58.8[a]	56.8[b]	49.4[d]	0.40	<0.01	<0.01	<0.01
EPD_8, %	41.6[c]	47.5[a]	45.6[b]	37.9[d]	0.40	<0.01	<0.01	<0.01
In situ rumen escape by EPD_5, kg/d	0.98[b]	0.85[c]	0.89[c]	1.04[a]	0.04	<0.01	0.04	0.04
In situ rumen escape by EPD_8, kg/d	1.20[a]	1.05[b]	1.10[b]	1.26[a]	0.04	<0.01	0.05	0.04
Estimated duodenal microbial protein flow based on EPD_5, kg/d	3.03	3.29	3.31	3.44	0.26	0.14	0.09	0.04
Estimated duodenal microbial protein flow based on EPD_8, kg/d	2.81	3.10	3.10	3.22	0.26	0.12	0.08	0.03

[1] HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets.

[2] *S*= Soluble fraction, *Pd*= Potentially degradable fraction, *D*= Potential degradability, k_d= Fractional rate of degradation of *Pd* (h^{-1}), ESD= Effective starch degradability calculated using a fractional rate of passage (k_p) of 0.05 h^{-1} (ESD_5) or 0.08 h^{-1} (ESD_8), EPD= Effective protein degradability calculated using a fractional rate of passage (k_p) of 0.05 h^{-1} (EPD_5) or 0.08 h^{-1} (EPD_8).

[3] Standard error of the mean for n = 4.

[a, b, c, d] indicate least-square means to differ within the row.

Table 11

Daily milk production and milk contents

	Experimental treatments[1]					*P*-value		
	HDcon	HDext	MDext	LDext	SEM[2]	Trt	HD × LMD	Con × Ext
Daily production:								
Milk, kg	30.0	30.2	30.2	29.8	3.0	0.98	0.85	0.96
Fat, kg	1.30	1.30	1.27	1.26	0.15	0.75	0.40	0.60
Protein, kg	1.01	0.99	0.99	0.98	0.10	0.87	0.61	0.53
Lactose, kg	1.42	1.43	1.43	1.42	0.15	0.96	0.91	0.89
ECM[3], kg	31.5	31.4	31.2	30.8	3.34	0.82	0.50	0.64
ECM, kg/kg DM intake	1.46	1.46	1.48	1.45	0.07	0.78	0.76	0.94
ECM, kg/kg concentrate intake	3.56^{ab}	3.67^{a}	3.53^{ab}	3.47^{b}	0.38	0.09	0.07	0.99
Milk composition:								
Fat, %	4.33	4.22	4.16	4.22	0.08	0.57	0.37	0.21
Protein, %	3.39	3.26	3.31	3.31	0.06	0.15	0.65	0.04
Lactose, %	4.74	4.68	4.73	4.73	0.03	0.36	0.53	0.36
Urea, mmol/L	5.30	4.66	4.68	5.24	0.34	0.22	0.94	0.18
FFA[4], mEq/L	0.39	0.46	0.41	0.42	0.08	0.82	0.82	0.46
SCC[5], x1000 cells/mL	70.3^{a}	53.2^{ab}	35.3^{b}	27.6^{b}	23.3	0.03^{T}	0.03^{T}	0.01^{T}

[1] HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets.

[2] SEM= Standard error of the mean for n = 4.

[3] ECM= Energy corrected milk.

[4] FFA= Free fatty acids.

[5] SCC= Somatic cell count.

[T] *P* values are for the $\log_{10}$ transformed variable.

[a, b] indicates least-square means to differ within the row.

Table 12

Excretion of nitrogen in feces, urine, and milk (N balance)

Item	Experimental treatments[1]				SEM[2]	*P*-Value		
	HDcon	HDext	MDext	LDext		Trt	HD × LMD	Con × Ext
N excreted (% of N intake)	88.9	88.6	88.0	89.3	1.66	0.95	0.95	0.86
Fecal N (% of excreted)	36.6	37.2	36.6	38.0	1.57	0.47	0.57	0.44
Urinary N (% of excreted)	30.1	29.2	29.2	29.9	2.60	0.81	0.89	0.51
Milk N (% of excreted)	33.3	33.6	34.3	32.1	1.60	0.50	0.78	0.96

[1] HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets.

[2] SEM= Standard error of the mean for n=4.

[a, b] indicate least-square means to differ within the row.

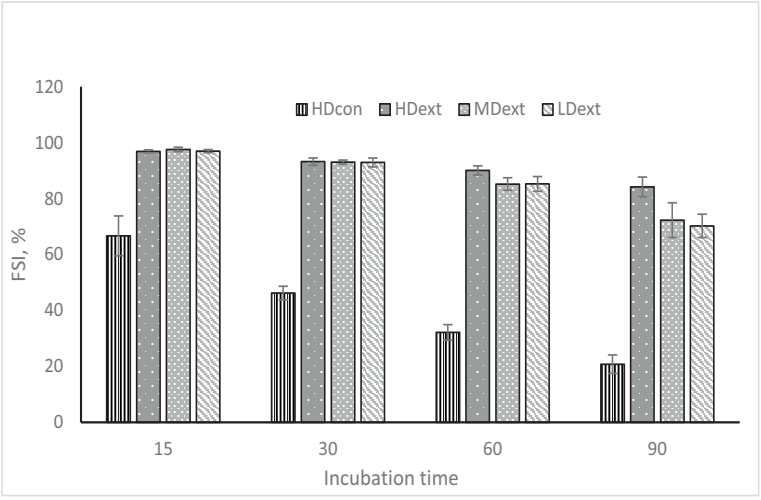


Figure 1. Fluid stability index (FSI) of experimental treatments (HDcon = High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded and LDext = Low-density extruded pellets) after 15, 30, 60 and 90 min of incubation in rumen fluid.

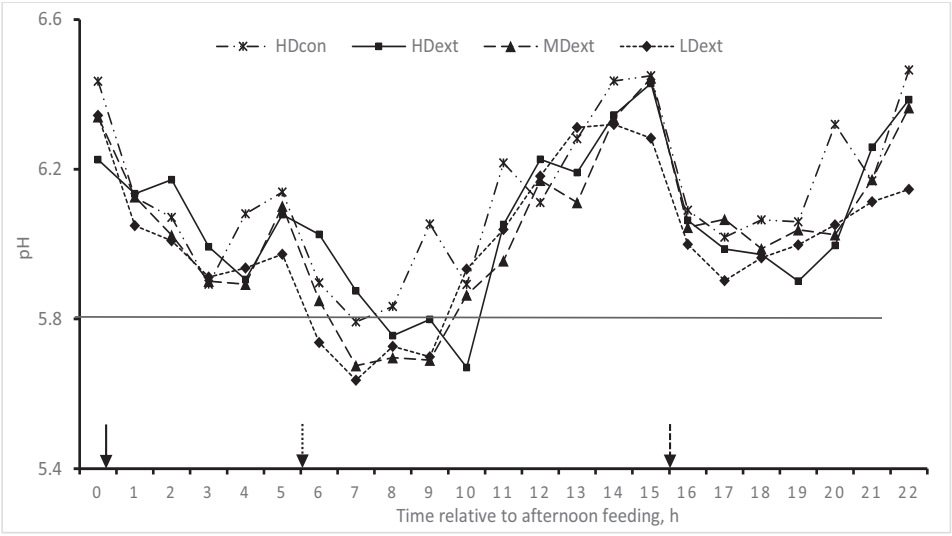


Figure 2. Diurnal rumen pH variation for experimental treatments (HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets). The solid arrow indicates afternoon feeding at 15:00, the dotted arrow for night feeding at 20:30, and the dashed arrow indicates morning feeding at 07:00.

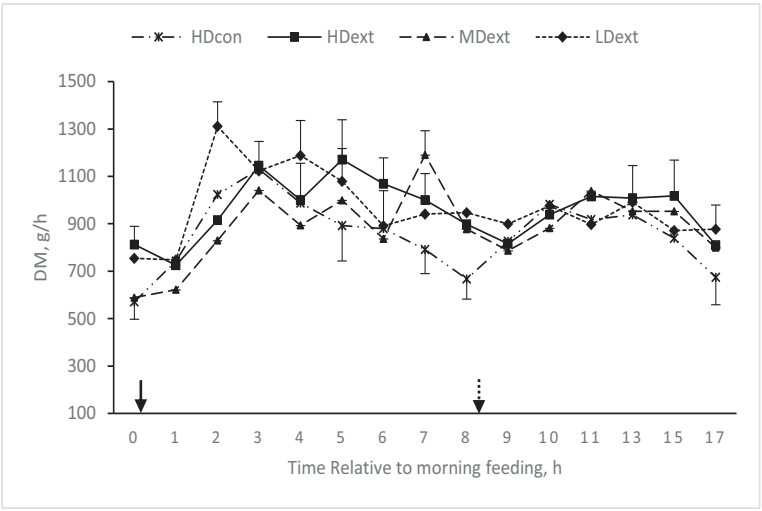
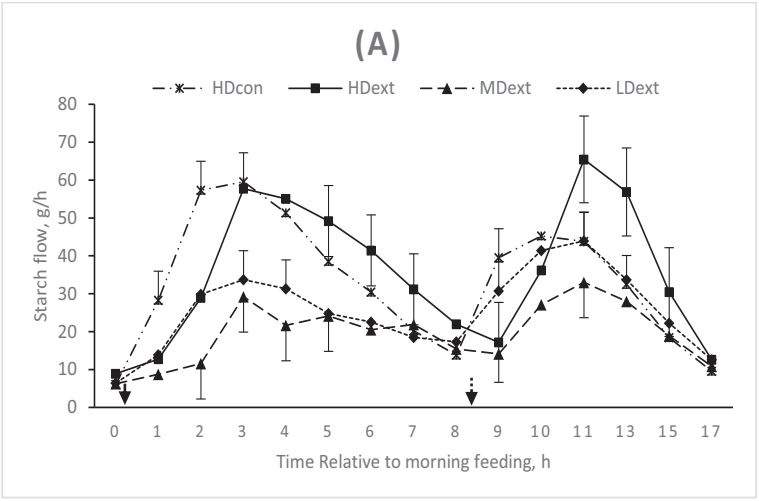


Figure 3. Postprandial duodenal DM flow for experimental treatments (HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets). The solid arrow indicates morning feeding, and the dashed arrow indicates afternoon feeding of experimental concentrates.



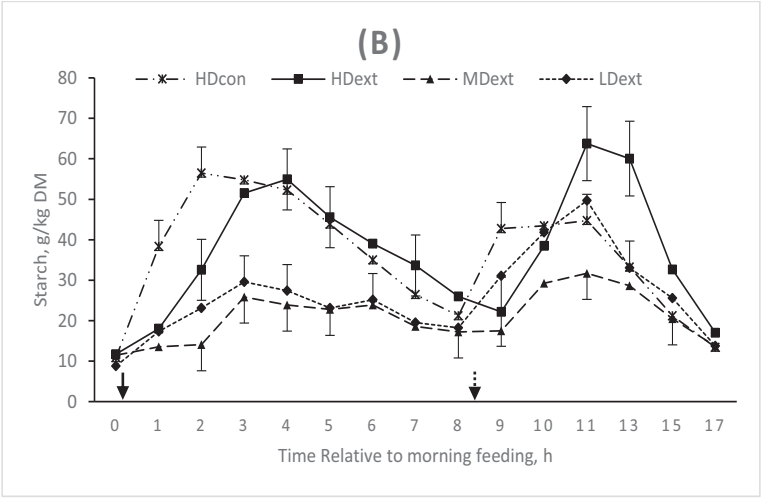
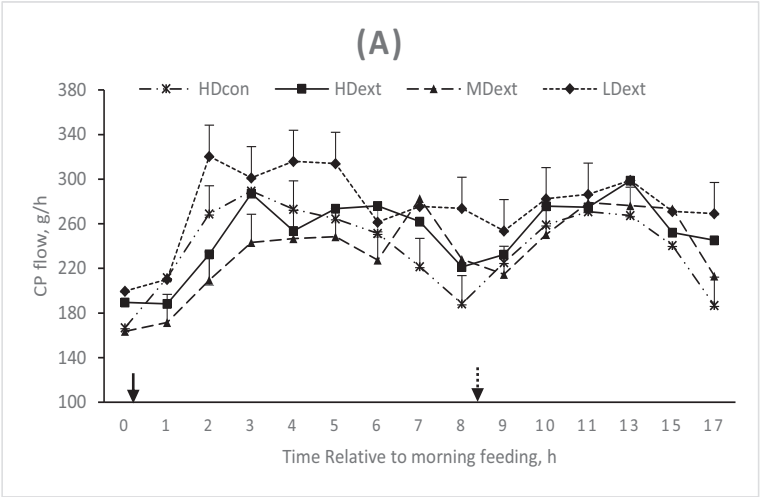


Figure 4. Postprandial duodenal flow (A) and concentration (B) of starch for experimental treatments (HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets). The solid arrow indicates morning feeding, and the dashed arrow indicates afternoon feeding of concentrates.



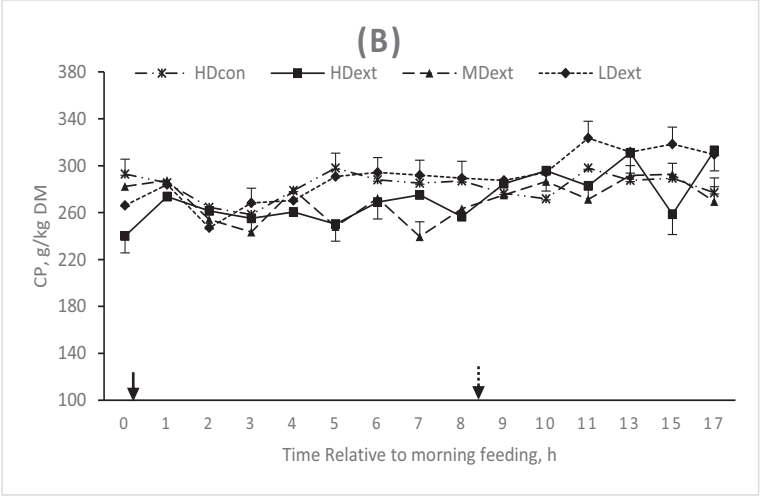


Figure 5. Postprandial duodenal flow (A) and concentration (B) of crude protein (CP) for experimental treatments (HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets). The solid arrow indicates morning feeding, and the dashed arrow indicates afternoon feeding of concentrate

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Appendix

Table A1

Coefficients of correlations (*P*-values in parenthesis) between independent[1] and dependent[2] variables and among dependent variables for all feeds (n=40) produced in Experiment 1.

	T3	T5	Torque	SME	DP	RE	BD	SV	SD	FSI
SH	0.023	-0.007	-0.053	-0.095	-0.343	-0.099	-0.068	-0.062	0.025	0.056
	(0.89)	(0.97)	(0.74)	(0.56)	(0.03)	(0.55)	(0.67)	(0.70)	(0.88)	(0.73)
SS	0.171	0.085	-0.301	0.552	-0.150	0.138	-0.195	-0.233	-0.161	0.034
	(0.29)	(0.60)	(0.06)	(<0.01)	(0.35)	(0.39)	(0.23)	(0.15)	(0.32)	(0.84)
C	-0.694	-0.933	0.156	0.102	0.267	-0.335	0.562	0.531	0.560	-0.013
	(<0.01)	(<0.01)	(0.34)	(0.53)	(0.10)	(0.03)	(<0.01)	(<0.01)	(<0.01)	(0.93)
T3		0.893	0.210	0.348	-0.383	0.657	-0.708	-0.604	-0.787	0.097
		(<0.01)	(0.19)	(0.03)	(0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	0.55
T5			0.014	0.106	-0.353	0.520	-0.664	-0.600	-0.709	0.043
			(0.93)	(0.52)	(0.02)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(0.79)
Torque				0.604	0.282	0.257	-0.115	-0.093	-0.238	0.187
				(<0.01)	(0.08)	(0.11)	(0.48)	(0.57)	(0.14)	(0.25)
SME					0.133	0.416	-0.343	-0.360	-0.412	0.255
					(0.41)	(0.01)	(0.03)	(0.02)	(0.01)	(0.11)
DP						-0.530	0.379	0.316	0.368	-0.346
						(<0.01)	(0.02)	(0.05)	(0.02)	(0.03)
RE							-0.809	-0.775	-0.873	0.591
							(<0.01)	(<0.01)	(<0.01)	(<0.01)
BD								0.923	0.959	-0.423
								(<0.01)	(<0.01)	(0.01)
SV									0.893	-0.560
									(<0.01)	(<0.01)
SD										-0.362
										(0.02)

[1] Processing conditions; Screen size in hammer mill (SH), Screw speed in extruder (SS), Cooling at the last section in extruder barrel (C).

[2] Extruder processing variables; Temperature in section 3 of extruder barrel (T3), Temperature in section 5 of extruder barrel (T5), Torque in extruder, Specific mechanical energy (SME), and Die pressure (DP). Physical properties of pellets; Radial expansion (RE), Bulk density (BD), Sinking velocity (SV), Specific density (SD), and Fluid stability index (FSI).

Table A2

Coefficients of correlations (*P*-values in parenthesis) between independent[1] and dependent[2] variables and among dependent variables for barley feeds (n=8) produced in Experiment 1.

	T3	T5	Torque	SME	DP	Exp	BD	SV	SD	FSI
SH	0.227	-0.052	-0.189	-0.320	-0.954	-0.802	-0.148	0.102	0.256	0.850
	(0.58)	(0.58)	(0.58)	(0.58)	(0.02)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)
SS	0.190	0.050	-0.114	0.852	-0.193	0.260	-0.519	-0.474	-0.412	0.327
	(0.65)	(0.91)	(0.79)	(0.01)	(0.65)	(0.53)	(0.19)	(0.24)	(0.31)	(0.43)
C	-0.922	-0.995	0.937	0.3835	0.219	-0.489	0.816	0.840	0.813	0.143
	(<0.01)	(<0.01)	(<0.01)	(0.35)	(0.60)	(0.21)	(0.01)	(0.01)	(0.01)	(0.74)
T3		0.926	-0.822	-0.099	-0.026	0.703	-0.867	-0.917	-0.955	-0.335
		(<0.01)	(0.01)	(0.82)	(0.95)	(0.05)	(<0.01)	(<0.01)	(<0.01)	(0.42)
T5			-0.942	-0.332	-0.176	0.5461	-0.818	-0.863	-0.832	-0.149
			(<0.01)	(0.42)	(0.68)	(0.16)	(0.01)	(0.01)	(0.01)	(0.72)
Torque				0.356	0.409	-0.360	0.819	0.828	0.734	-0.114
				(0.39)	(0.31)	(0.38)	(0.01)	(0.01)	(0.04)	(0.79)
SME					0.219	0.270	-0.102	-0.115	-0.134	0.024
					(0.60)	(0.51)	(0.81)	(0.79)	(0.75)	(0.96)
DP						0.615	0.421	0.180	0.011	-0.838
						(0.10)	(0.30)	(0.67)	(0.98)	(0.01)
Exp							-0.428	-0.646	-0.755	-0.660
							(0.29)	(0.08)	(0.03)	(0.07)
BD								0.936	0.897	-0.116
								(<0.01)	(<0.01)	(0.78)
SV									0.963	0.033
									(<0.01)	(0.94)
SD										0.248
										(0.55)

[1] Processing conditions; Screen size in hammer mill (SH), Screw speed in extruder (SS), Cooling at the last section in extruder barrel (C).

[2] Extruder processing variables; Temperature in section 3 of extruder barrel (T3), Temperature in section 5 of extruder barrel (T5), Torque in extruder, Specific mechanical energy (SME) and Die pressure (DP). Physical properties of pellets; Radial expansion (RE), Bulk density (BD), Sinking velocity (SV), Specific density (SD), and Fluid stability index (FSI).

Table A3

Coefficients of correlations (*P*-values in parenthesis) between independent[1] and dependent[2] variables and among dependent variables for maize feeds (n=8) produced in Experiment 1.

	T3	T5	Torque	SME	DP	Exp	BD	SV	SD	FSI
SH	0.076	0.064	0.586	0.441	-0.201	-0.020	-0.094	-0.110	-0.062	-0.339
	(0.86)	(0.88)	(0.13)	(0.27)	(0.63)	(0.96)	(0.82)	(0.80)	(0.88)	(0.41)
SS	0.202	0.102	-0.666	0.778	-0.513	0.367	-0.206	-0.188	-0.225	-0.370
	(0.63)	(0.81)	(0.07)	(0.02)	(0.19)	(0.37)	(0.63)	(0.66)	(0.59)	(0.37)
C	-0.961	-0.985	-0.172	-0.337	0.826	-0.922	0.972	0.940	0.971	0.627
	(<0.01)	(<0.01)	(0.68)	(0.41)	(0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(0.10)
T3		0.993	0.134	0.531	-0.916	0.977	-0.981	-0.918	-0.990	-0.774
		(<0.01)	(0.75)	(0.18)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(0.02)
T5			0.182	0.449	-0.882	0.956	-0.985	-0.934	-0.989	-0.716
			(0.67)	(0.26)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(0.05)
Torque				-0.108	0.099	-0.050	-0.079	-0.066	-0.069	-0.233
				(0.80)	(0.82)	(0.91)	(0.85)	(0.88)	(0.87)	(0.58)
SME					-0.736	0.614	-0.521	-0.511	-0.532	-0.768
					(0.04)	(0.11)	(0.19)	(0.20)	(0.18)	(0.03)
DP						-0.941	0.930	0.888	0.932	0.745
						(<0.01)	(<0.01)	(<0.01)	(<0.01)	(0.03)
Exp							-0.967	-0.913	-0.980	-0.775
							(<0.01)	(<0.01)	(<0.01)	(0.02)
BD								0.965	0.997	0.703
								(<0.01)	(<0.01)	(0.05)
SV									0.956	0.582
									(<0.01)	(0.13)
SD										0.724
										(0.04)

[1] Processing conditions; Screen size in hammer mill (SH), Screw speed in extruder (SS), Cooling at the last section in extruder barrel (C).

[2] Extruder processing variables; Temperature in section 3 of extruder barrel (T3), Temperature in section 5 of extruder barrel (T5), Torque in extruder, Specific mechanical energy (SME) and Die pressure (DP). Physical properties of pellets; Radial expansion (RE), Bulk density (BD), Sinking velocity (SV), Specific density (SD), and Fluid stability index (FSI).

Table A4

Coefficients of correlations (*P*-values in parenthesis) between independent[1] and dependent[2] variables and among dependent variables for soybean meal (SBM) feeds (n=8) produced in Experiment 1.

	T3	T5	Torque	SME	DP	Exp	BD	SV	SD	FSI
SH	-0.063	-0.007	0.157	0.106	0.183	0.000	0.081	-0.258	0.553	0.242
	(0.88)	(0.99)	(0.71)	(0.80)	(0.66)	(1.00)	(0.85)	(0.54)	(0.15)	(0.56)
SS	0.188	0.064	-0.734	0.877	-0.305	-0.750	-0.340	-0.258	0.158	0.697
	(0.66)	(0.88)	(0.04)	(<0.01)	(0.46)	(0.03)	(0.41)	(0.54)	(0.71)	(0.05)
C	-0.971	-0.986	0.564	0.452	0.916	-0.500	0.921	0.775	0.632	0.272
	(<0.01)	(<0.01)	(0.15)	(0.26)	(<0.01)	(0.21)	(<0.01)	(0.02)	(0.09)	(0.51)
T3		0.989	-0.656	-0.270	-0.945	0.329	-0.963	-0.752	-0.674	-0.085
		(<0.01)	(0.08)	(0.52)	(<0.01)	(0.43)	(<0.01)	(0.03)	(0.07)	(0.84)
T5			-0.560	-0.377	-0.917	0.426	-0.921	-0.742	-0.677	-0.158
			(0.15)	(0.36)	(<0.01)	(0.29)	(<0.01)	(0.04)	(0.07)	(0.71)
Torque				-0.334	0.810	0.166	0.789	0.659	0.230	-0.143
				(0.42)	(0.01)	(0.70)	(0.02)	(0.08)	(0.58)	(0.74)
SME					0.179	-0.930	0.126	0.137	0.475	0.800
					(0.67)	(<0.01)	(0.77)	(0.75)	(0.23)	(0.02)
DP						-0.275	0.944	0.757	0.608	0.149
						(0.51)	(<0.01)	(0.03)	(0.11)	(0.72)
Exp							-0.201	-0.258	-0.474	-0.775
							(0.63)	(0.54)	(0.24)	(0.02)
BD								0.755	0.546	0.059
								(0.03)	(0.16)	(0.89)
SV									0.327	-0.070
									(0.43)	(0.87)
SD										0.167
										(0.69)

[1] Processing conditions; Screen size in hammer mill (SH), Screw speed in extruder (SS), Cooling at the last section in extruder barrel (C).

[2] Extruder processing variables; Temperature in section 3 of extruder barrel (T3), Temperature in section 5 of extruder barrel (T5), Torque in extruder, Specific mechanical energy (SME) and Die pressure (DP). Physical properties of pellets; Radial expansion (RE), Bulk density (BD), Sinking velocity (SV), Specific density (SD), and Fluid stability index (FSI).

Table A5

Coefficients of correlations (*P*-values in parenthesis) between independent[1] and dependent[2] variables and among dependent variables for barley+soybean meal (B+SBM; 50:50) feeds (n=8) produced in Experiment 1.

	T3	T5	Torque	SME	DP	Exp	BD	SV	SD	FSI
SH	-0.044	-0.117	-0.801	-0.500	-0.916	-0.606	-0.280	0.086	0.268	0.615
	(0.92)	(0.78)	(0.02)	(0.21)	(<0.01)	(0.11)	(0.50)	(0.84)	(0.52)	(0.10)
SS	0.479	0.139	-0.376	0.819	-0.061	0.501	-0.414	-0.571	-0.219	0.467
	(0.23)	(0.74)	(0.36)	(0.01)	(0.89)	(0.21)	(0.31)	(0.14)	(0.60)	(0.24)
C	-0.741	-0.970	0.261	0.138	0.346	-0.501	0.800	0.699	0.830	-0.177
	(0.04)	(<0.01)	(0.53)	(0.74)	(0.40)	(0.21)	(0.02)	(0.05)	(0.01)	(0.68)
T3		0.850	-0.382	0.318	-0.240	0.798	-0.923	-0.959	-0.846	0.496
		(0.01)	(0.35)	(0.44)	(0.57)	(0.02)	(<0.01)	(<0.01)	(0.01)	(0.21)
T5			-0.188	0.062	-0.236	0.670	-0.837	-0.823	-0.911	0.180
			(0.66)	(0.88)	(0.57)	(0.07)	(0.01)	(0.01)	(<0.01)	(0.67)
Torque				0.217	0.847	0.125	0.636	0.324	0.081	-0.874
				(0.61)	(0.01)	(0.77)	(0.09)	(0.43)	(0.85)	(<0.01)
SME					0.445	0.645	-0.082	-0.429	-0.225	-0.008
					(0.27)	(0.08)	(0.85)	(0.29)	(0.59)	(0.99)
DP						0.340	0.564	0.159	0.093	-0.717
						(0.41)	(0.15)	(0.71)	(0.83)	(0.05)
Exp							-0.557	-0.800	-0.810	0.115
							(0.15)	(0.02)	(0.01)	(0.79)
BD								0.879	0.791	-0.657
								(<0.01)	(0.02)	(0.08)
SV									0.815	-0.374
									(0.01)	(0.36)
SD										-0.243
										(0.56)

[1] Processing conditions; Screen size in hammer mill (SH), Screw speed in extruder (SS), Cooling at the last section in extruder barrel (C).

[2] Extruder processing variables; Temperature in section 3 of extruder barrel (T3), Temperature in section 5 of extruder barrel (T5), Torque in extruder, Specific mechanical energy (SME) and Die pressure (DP). Physical properties of pellets; Radial expansion (RE), Bulk density (BD), Sinking velocity (SV), Specific density (SD), and Fluid stability index (FSI).

Table A6

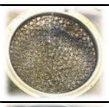
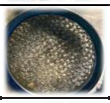
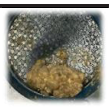
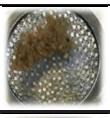
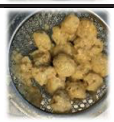
Coefficients of correlations (*P*-values in parenthesis) between independent[1] and dependent[2] variables and among dependent variables for maize+soybean meal (M+SBM; 50:50) feeds (n=8) produced in Experiment 1.

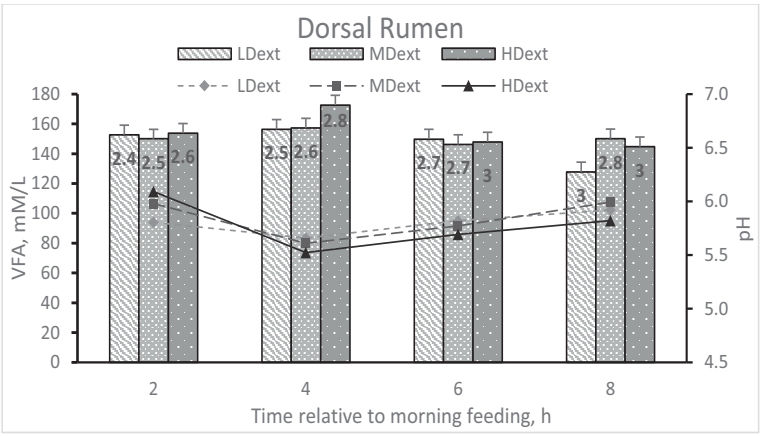
	T3	T5	Torque	SME	DP	Exp	BD	SV	SD	FSI
SH	0.719	0.045	-0.376	-0.448	-0.765	-0.108	-0.147	-0.550	-0.429	0.553
	(0.04)	(0.92)	(0.36)	(0.27)	(0.03)	(0.80)	(0.73)	(0.16)	(0.29)	(0.15)
SS	0.166	0.112	-0.849	0.731	-0.348	0.181	-0.532	-0.527	-0.376	0.287
	(0.69)	(0.79)	(0.01)	(0.04)	(0.40)	(0.67)	(0.17)	(0.18)	(0.36)	(0.49)
C	-0.608	-0.987	0.000	0.024	0.209	-0.903	0.720	0.538	0.644	-0.668
	(0.11)	(<0.01)	(1.00)	(0.96)	(0.62)	(<0.01)	(0.04)	(0.17)	(0.08)	(0.07)
T3		0.671	-0.390	-0.218	-0.780	0.597	-0.702	-0.896	-0.816	0.885
		(0.07)	(0.34)	(0.60)	(0.02)	(0.12)	(0.05)	(<0.01)	(0.01)	(<0.01)
T5			-0.128	0.010	-0.313	0.931	-0.815	-0.650	-0.724	0.744
			(0.76)	(0.98)	(0.45)	(<0.01)	(0.01)	(0.08)	(0.04)	(0.03)
Torque				-0.268	0.706	-0.140	0.512	0.669	0.403	-0.562
				(0.52)	(0.05)	(0.74)	(0.19)	(0.07)	(0.32)	(0.15)
SME					0.267	0.079	-0.288	-0.082	-0.168	-0.210
					(0.52)	(0.85)	(0.49)	(0.85)	(0.69)	(0.62)
DP						-0.269	0.553	0.831	0.563	-0.865
						(0.52)	(0.16)	(0.01)	(0.15)	(0.01)
Exp							-0.782	-0.614	-0.587	0.677
							(0.02)	(0.11)	(0.13)	(0.07)
BD								0.888	0.898	-0.791
								(<0.01)	(<0.01)	(0.02)
SV									0.885	-0.902
									(<0.01)	(<0.01)
SD										-0.739
										(0.04)

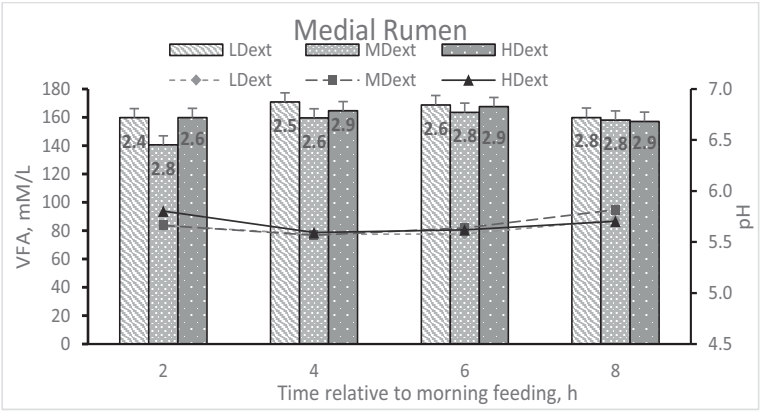
[1] Processing conditions; Screen size in hammer mill (SH), Screw speed in extruder (SS), Cooling at the last section in extruder barrel (C).

[2] Extruder processing variables; Temperature in section 3 of extruder barrel (T3), Temperature in section 5 of extruder barrel (T5), Torque in extruder, Specific mechanical energy (SME) and Die pressure (DP). Physical properties of pellets; Radial expansion (RE), Bulk density (BD), Sinking velocity (SV), Specific density (SD), and Fluid stability index (FSI).

Table A7. Visual inspection of fluid stability of feed pellets produced in Experiment 1. State (before drying) of pellets after 90 min incubation in rumen fluid at 39 °C. NA, pic not available.

Screen Size	Die Size	Screw Speed	Cooling	Barley	Maize	Barley+ SBM	Maize+ SBM
2	3	210	No			NA	
			Yes			NA	
		300	No			NA	
			Yes			NA	
	6	210	No			NA	
			Yes			NA	
		300	No			NA	
			Yes			NA	
6		210	No			NA	
			Yes			NA	
		300	No			NA	
			Yes				





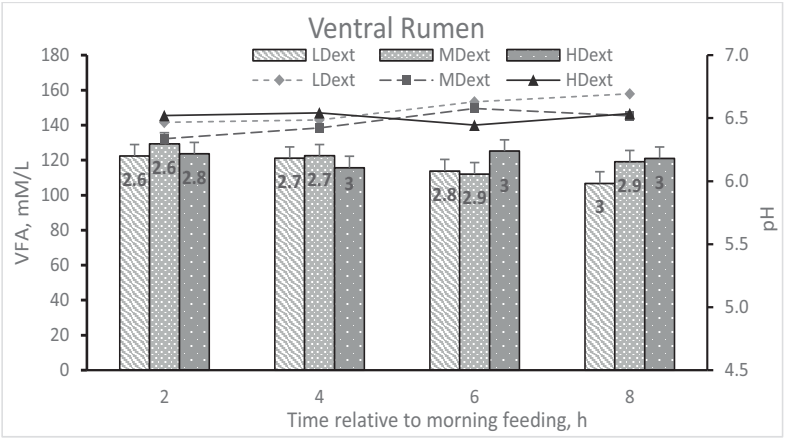


Figure A1. Postprandial production of VFA (bars), pH (lines) and acetate:propionate ratio (numbers inside bars) at three different locations in the rumen for extruded pellets with low-density (LD), medium-density (MD), and high-density (HD) used in Paper-II

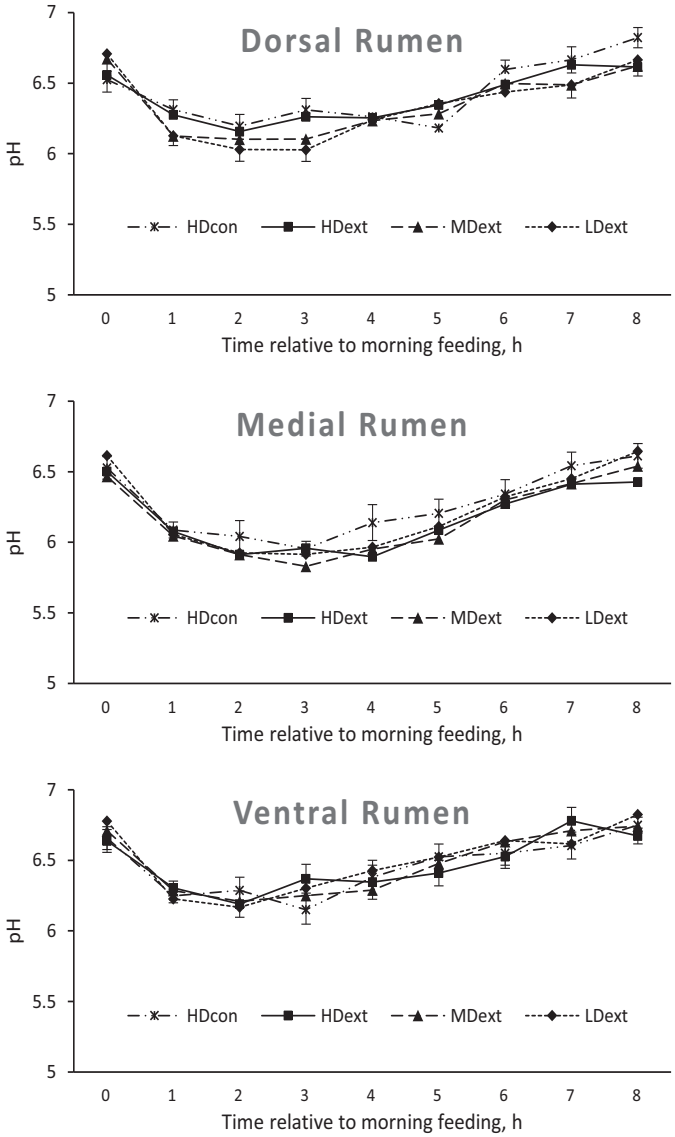


Figure A2. Postprandial rumen pH at three different locations in the rumen for the experimental treatments (HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets) used in Paper-III

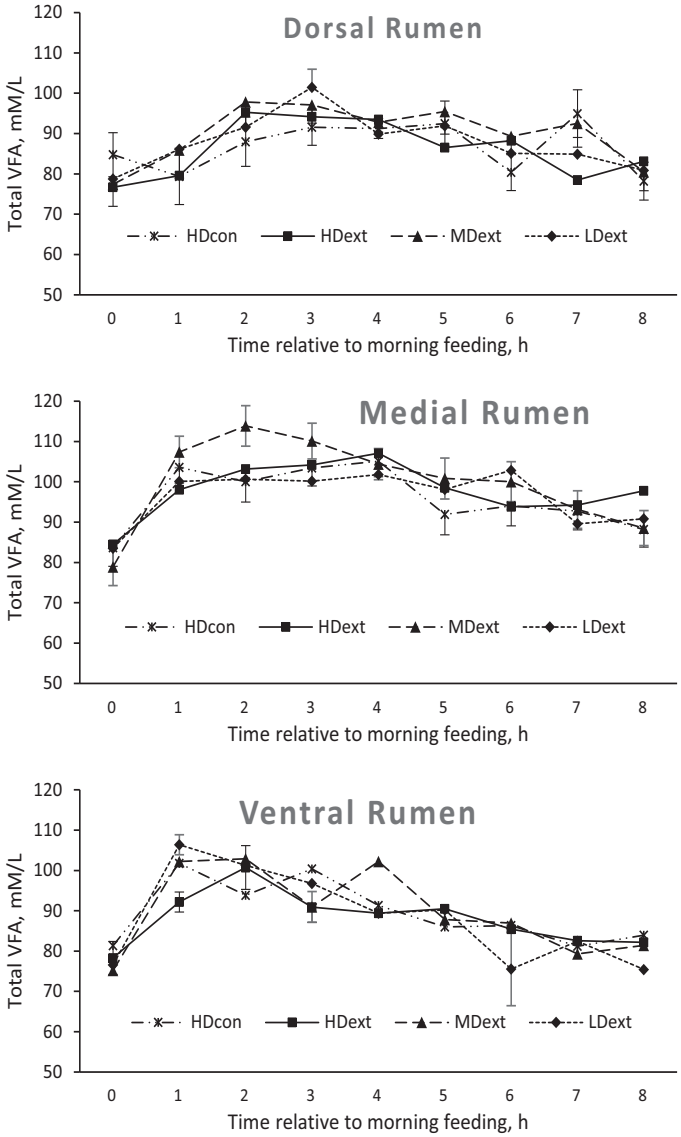


Figure A3. Postprandial total VFA concentration in rumen fluid taken from three different locations in the rumen for the experimental treatments (HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets) used in Paper-III

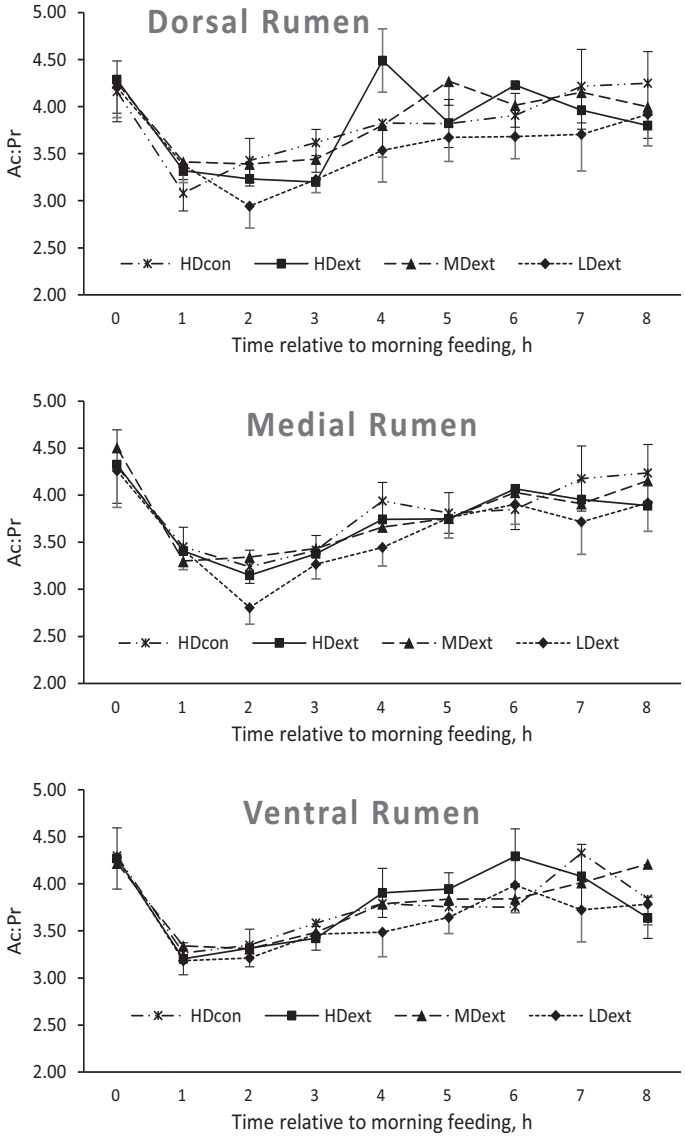


Figure A4. Postprandial acetate:propionate (Ac:Pr) ratio in rumen fluid taken from three different locations in the rumen for the experimental treatments (HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets) used in Paper-III

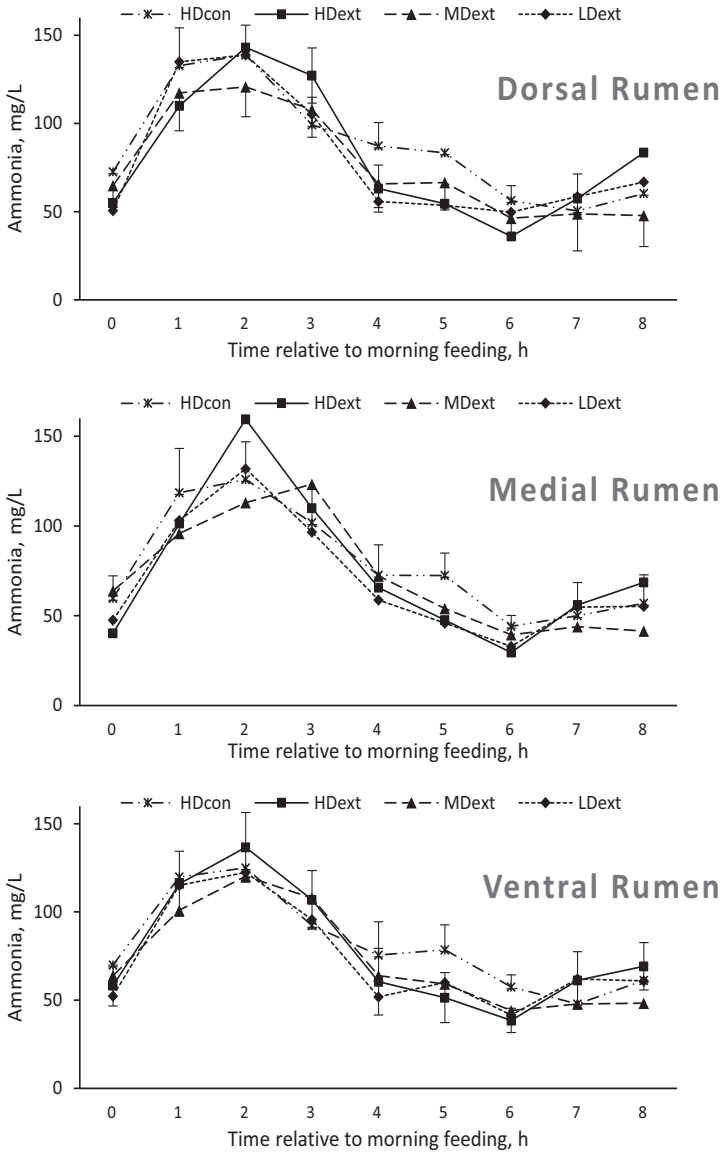


Figure A5. Postprandial ammonia concentration in rumen fluid taken from three different locations in the rumen for the experimental treatments (HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets) used in Paper-III

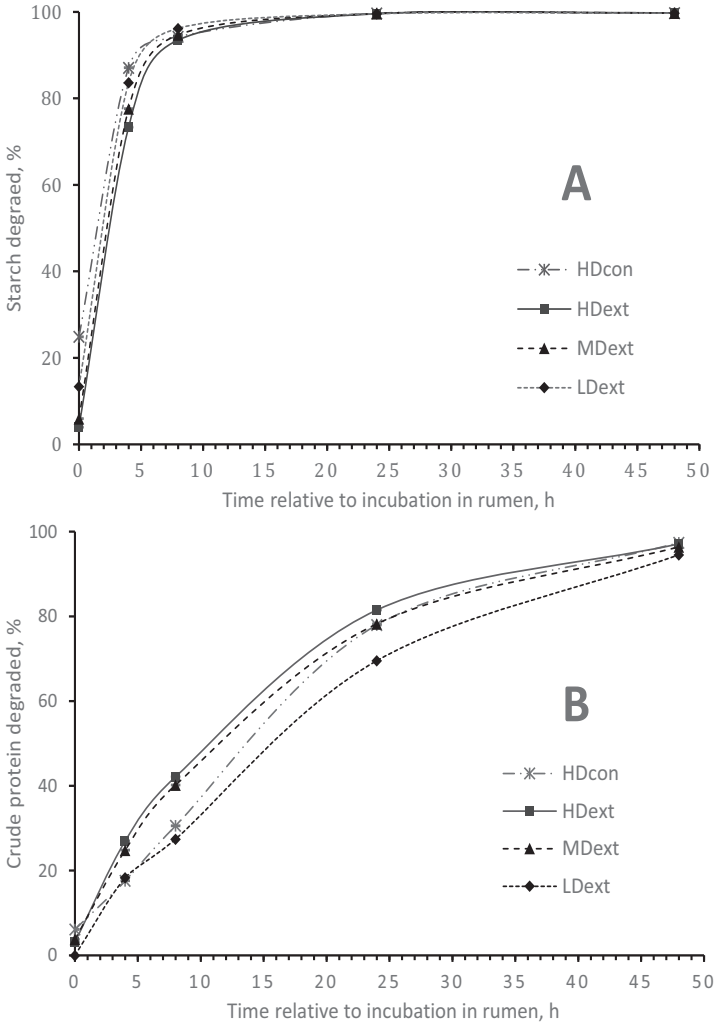


Figure A6. *In situ* rumen degradation profiles of starch (A) and crude protein (B) for the experimental treatments (HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets) used in Paper-III.

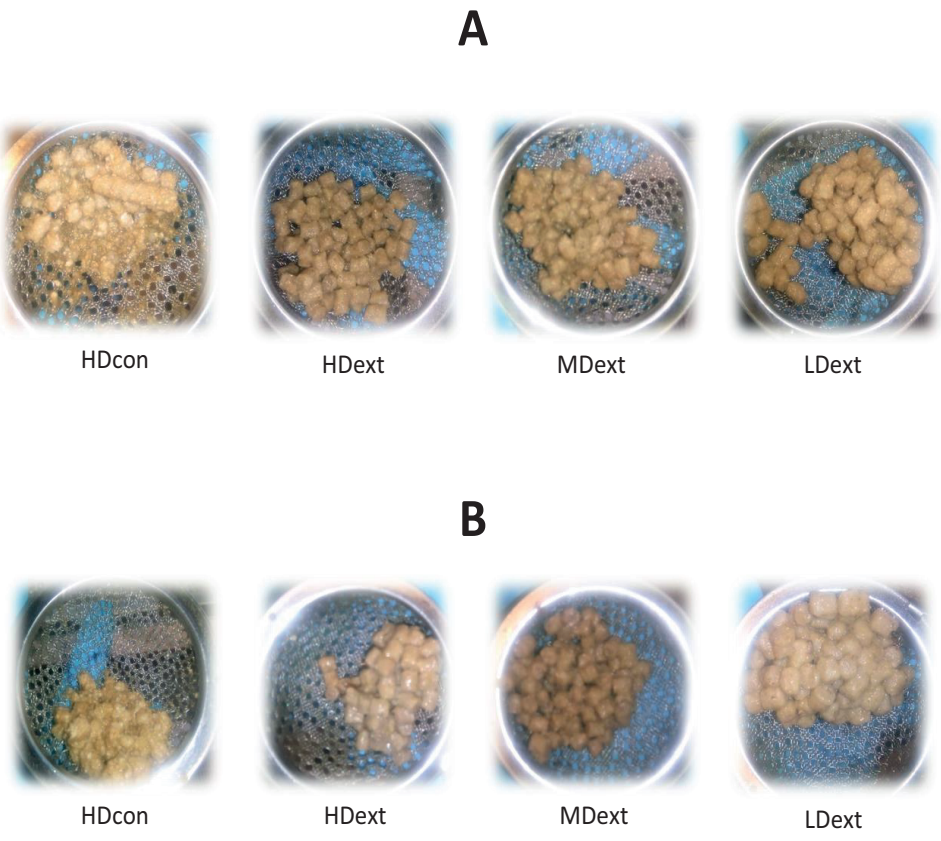


Figure A7. Visual inspection of fluid stability of the experimental treatments (HDcon= High-density conventional, HDext = High-density extruded, MDext = Medium-density extruded, LDext = Low-density extruded pellets) used in Paper-III. State (before drying) of feed pellets after 15 min (A) and 30 min (B) incubation in rumen fluid at 39 °C.

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